DOCUMENT RESUME

ED 364 033	EC 302 603

AUTHOR	Hagerman, Randi Jenssen, Ed.; McKenzie, Pamela, Ed.
TITLE	International Fragile X Conference Proceedings (3rd, Snowmass/Aspen, Colorado, 1992).
INSTITUTION	National Fragile X Foundation, Denver, CO.
REPORT NO	ISBN-0-915667-22-3
PUB DATE	92
NOTE	399p.
AVAILABLE FROM	National Fragile X Foundation, 1441 York Street, Suite 215, Denver, CO 80206 ($26.95; 10 or more $20 each plus shipping and handling).
PUB TYPE	Collected Works - Conference Proceedings (021)
EDRS PRICE	MF01 Plus Postage. PC Not Available from EDRS.
DESCRIPTORS	*Clinical Diagnosis; *Congenital Impairments; Diagnostic Tests; *Educational Methods; Educational Needs; Family Counseling; *Genetics; Individual Characteristics; Intervention; Learning Disabilities; Medical Evaluation; *Mental Retardation; Multiple Disabilities; Sex Differences; Special Health Problems; *Symptoms (Individual Disorders); Therapy
IDENTIFIERS	*Fragile X Syndrome

ABSTRACT

This proceedings document presents the texts or summaries of 52 papers given at a 1992 conference on Fragile X syndrome. Preliminary information includes names and institutional affiliations of conference faculty, information about the National Fragile X Foundation, awards presented at the conference, and a list of resource centers (by state). Papers are organized into the following four categories: (1) physical, cognitive, and behavioral phenotype and medical follow up (e.g., medical management, physical features, seizures, oculovisual dysfunction, learning disability, socioemotional characteristics of adult mentally retarded men with Fragile X syndrome, behavioral assessments, and psychosis); (2) educational and behavioral interventions (e.g., the Fragile X Educational Assistance Program, combined occupational and speech therapy, assistive technology, psychotherapy, vocational training, and facilitating a parent/family support group; (3) cytogenetic and molecular diagnostic testing (e.g., molecular genetics, the genetic lesion causing the disorder, genetic counseling, prenatal diagnosis, cytogenetics, prevalence of chromosomal abnormalities, and carrier testing of persons with suggestive family histories); and (4) social concerns (screening, economic impact of the Fragile X syndrome in Colorado, and ethical issues in the provision of genetic services). Papers include references. (DB)

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1992 INTERNATIONAL FRAGILE X CONFERENCE PROCEEDINGS

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Edited by
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and
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The National Fragile X Foundation
Promoting education and research

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Printed in the United States of America

ISBN # 0-915667-22-3

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SPECTRA PUBLISHING CO., INC
P. O. BOX 1403
DILLON, COLORADO 80435
(303) 468-6439

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DEDICATION

This book is dedicated to the fragile X children and their families who have taught us what we know and to the National Fragile X Foundation board members of the past and future whose faith, enthusiasm, and generosity continue to facilitate conferences captured in these proceedings.

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PREFACE

The publication of these proceedings was made possible by a grant from the Frost Foundation. We are very grateful for their generous support.

The fragile X field is changing rapidly and effective communication is our only means of keeping pace with the changes. The National Fragile X Foundation was founded in 1984 to promote communication. We needed it then because no one knew about fragile X and we had to get information out to the public, the professionals and the families. Now the information is even more technical and it is of general interest to a broad range of professionals. The discoveries have uncovered a new mechanism of mutation, a new understanding of the molecular basis of cognitive, emotional and behavioral problems and new treatments. The recent developments required nothing less than an international conference which took place June 16-20, 1992. It was flooded with faculty who are the leaders in their fields and who were dedicated to educating us all in one of the loveliest and loftiest locations in the world, Snowmass/Aspen, Colorado. We thank them, for they came with inadequate compensation and they donated their manuscripts for the sake of education. These proceedings represent approximately seventy percent of the information presented at the conference. There was also a range of technical information presented, so the proceedings are divided into sections for fast reference.

Funding continues to be a struggle. The support from Joslins in Denver was remarkable. We gratefully recognized their dedication to the Fragile X Project with an award to Joslins, which was accepted by Mrs. Chabot. We also thank Michael Gronquist from Mead Johnson Pharmaceuticals, Abbot Laboratories, Vivigen Laboratories, the Colorado Developmental Disabilities Planning Council, and the National Institute of Child Health and Development (NICHD) grant #HD30074-01 for their support of the conference. We are most appreciative of Jessica White, MSS, CMP, and her staff from the Conference Planning & Education Services Department at The Children's Hospital, who organized the details and did an exceptional job. As always, the staff of the Child Development Unit, including Claire Hull, Mela Ramirez, Joan Gillis and Sherrill Harris, were ever supportive and we are grateful. Sue Foster of Pro-Word Solutions provided efficient and excellent secretarial support and assisted in editing and formatting all of the manuscripts for publication. Robert Wyler, a former board member, is our publisher and ever supportive of fragile X work. Finally, our Executive Director, Sabrina Jewell-Smart, was recognized as a gracious host but most importantly, an astute leader who will take this Foundation far into the future to help fragile X children and their families.

Randi Hagerman, MD
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August 1992
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The National Fragile X Foundation was incorporated in 1984. Its mission is to promote education and research regarding the fragile X syndrome and other X-linked causes of mental retardation. One goal of the Foundation is to facilitate an educational program resulting in appropriate diagnosis, medical management, and education for every fragile X family. Research in the field of genetics is burgeoning and fragile X is at the forefront. The Foundation fosters further work in all related areas.

The Foundation has produced an informative newsletter since its inception and encourages networking through over 50 Resource Centers of families and professionals. It has sponsored three international conferences.

When families contact the Foundation after receiving a diagnosis of fragile X, we not only provide them with information and referrals to appropriate resources, but also help them to realize that the current knowledge of fragile X was obtained in only the last few years. In the past decade the rate and accuracy of diagnosis has improved, successful interventions were developed, and the gene was identified. If such steps can be accomplished in just a few years, who is to say a cure isn't obtainable in the near future?

In order to continue these activities and more, an international public awareness campaign is planned, but can only be done with adequate funding which is the Board's current goal.

Last year the economic impact of fragile X was studied by Lauria et al. (these proceedings) from the University of Colorado. His findings indicate the combined cost of care for a severely affected individual from birth to 72 years of age is 1.9 million dollars. Given a 1990 population of 3.3 million in Colorado and an average male penetrance of 1 in 1000 and 1 in 2000 for females, the annual cost of state provided care is over 73 million dollars. Nationally, the impact equals over 4 billion dollars annually to provide care for the severely affected fragile X population! Obviously this figure does not represent the educational dollars spent each year to provide the necessary special education and therapies to the less affected population in the public schools. The amount of money being expended on individuals with fragile X, not to mention the vast amounts invested in research, demonstrate the significance of this disorder.

In the future, social and public policy will be determined by what is known about fragile X. The genetics and ethics of disease are current issues. The Foundation with its network wants to be at the forefront when changes occur and policies determined that will affect all our lives.

The Third International Fragile X Conference provided a forum of suggestions and critiques from the families and professionals who attended. The recommendations include: to formalize the Resource Center Network; to implement a public awareness campaign, to fund fellowships and further research, and to provide workshops and community in-service programs. This feedback will be used to give direction both short and long term to The National Fragile X Foundation.

The Foundation is a non-profit organization and is dependent upon grants and donations to continue its existence. We feel that our mission, to promote education, support for families and research, is very important and provides a much needed service. Short term goals are to establish and support functional Resource Centers in every population area, to develop an international network of similar organizations, to begin a massive public awareness campaign, and to secure future funding sources. The National Fragile X Foundation needs your support!

AWARDS PRESENTED BY THE NATIONAL FRAGILE X FOUNDATION

at the

1992 THIRD INTERNATIONAL FRAGILE X CONFERENCE
SNOWMASS/ASPEN

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presented to
GENE AND WALTER KOELBEL

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presented to
RANDI JENSSEN HAGERMAN, MD

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I. Physical, Cognitive & Behavioral Phenotype and Medical Follow-Up

OVERVIEW OF CONFERENCE:
THE CLINICAL PHENOTYPE AND TREATMENT

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INTRODUCTION

The fragile X field has changed considerably within the last year with the advances in molecular biology. The identification of the FMR-1 gene brings a molecular basis for the spectrum of involvement seen clinically (Verkerk et al., 1991; Yu et al., 1991; Rousseau et al., 1991). The gene includes a CGG sequence which amplifies dramatically leading to methylation such that the FMR-1 protein is not produced in most affected individuals (Pieretti et al., 1991).

Very little is known about the FMR-1 protein and why its absence causes both intellectual problems and the physical findings of a long face, prominent ears, flat feet, loose joints and macroorchidism. Researchers are in the midst of developing an understanding of molecular-clinical correlations and widespread DNA testing at several centers has yielded some interesting results (Rousseau et al., 1991; Snow et al., 1992; Rousseau, 1992; Brown, 1992; Hagerman, 1992). First, there is more than one way to eliminate FMR-1 protein production. Hirst (these proceedings) and Wohrle et al. (1992) reported the fragile X syndrome phenotype in a male with a deletion at the Xq27.3 location which included the FMR-1 gene. Second, not all patients with cytogenetic expression of the fragile site have the CGG amplification in the FMR-1 gene. Approximately 2% to 5% of previously diagnosed fragile X syndrome patients are negative by direct DNA probe and PCR testing for the CGG amplification (Hagerman, 1992). Although some patients have the common fragile site at Xq27.2 (Sutherland & Baker, 1990), others have different mutations which disrupt chromatin packaging and cause a similar fragile site. One of these mutations is FRAXE which is distal to FMR-1 but may be associated with intellectual deficits (Hirst, these proceedings, and Sutherland & Baker, 1992). Perhaps a family of mutations will be identified in this region, although clearly the FMR-1 CGG amplification is the most common. It behooves us to focus on the physical and behavioral phenotype because these features will help us distinguish other variant subgroups to correlate with divergent molecular findings.

An analysis of the spectrum of involvement behaviorally, physically and intellectually furthers our understanding of the clinical relevance of molecular variations of the FMR-1 gene. Perhaps these variations will affect the prognosis of an individual with fragile X syndrome. The variability which is seen in FMR-1 includes the length of CGG amplification which can vary from a small insert of 54 to 200 repetitive sequences to a full smear of 600 to approximately

1,000 repeats. In addition, a mosaic pattern which has a small insert in some cells but a smear in other cells is also seen. When the amplification is large, methylation occurs which apparently shuts off the FMR-1 gene and protein production. Lastly, in females, X-inactivation occurs presumably in a random fashion. However, whether the fragile X chromosome is inactivated or the normal X chromosome is inactivated in a woman with a large CGG amplification may have important clinical consequences (Meltesen et al., these proceedings).

The relative significance of these molecular variations will be sorted out over the next few months to years, although the bottom line appears to be how much FMR-1 protein is produced. Individuals with a mosaic pattern including a subpopulation of cells with a small insert which produces adequate amounts of protein theoretically should be better off clinically than those with a full mutation and no protein production. There have been anecdotal reports of higher functioning mosaic males and Staley et al. (1992) report a significant IQ difference between mosaic males and full mutation males. However, other reports have shown no significant difference between mosaics and full mutation males (Rousseau, 1992; deVries et al., 1992). Perhaps a threshold effect exists where a given level of protein production is necessary to significantly alleviate the symptoms of fragile X syndrome. A brief review of physical and behavioral features follows.

THE PHYSICAL PHENOTYPE

The classical triad of features in the male includes large or prominent ears, a long face and macroorchidism. Approximately 80% to 90% of fragile X males demonstrate one or more of these features after puberty. The prepubertal male is more likely to present with prominent ears and hyperextensible finger joints. Early studies suggested a connective tissue dysplasia in this syndrome (Opitz et al, 1984, Hagerman et al., 1984), but very little work has been done to confirm or reject this observation. Waldstein et al. (1986) have reported thin and sparse elastin fibers in the dermis and in the cardiac valves (Waldstein & Hagerman, 1988), but the relationship between this finding and the FMR-1 protein is not known. Nevertheless, other physical features that are related to looseness of the connective tissue are common, including pectus excavatum, mitral valve prolapse, soft velvety skin, puffy eyelids, double-jointed thumbs and flat feet (Table 1).

In addition, more significant malformations such as club foot or cleft palate are seen in approximately 5% of fragile X patients and may also be related to a connective tissue dysplasia (Hagerman, 1991). The macroorchidism appears to be unrelated to the connective tissue dysplasia, although CNS/hypothalamic dysfunction has been postulated since the testicular size dramatically increases in early puberty (Butler et al., 1992; Fryns, et al., 1987).

In female heterozygotes the spectrum of involvement is much broader and a significant proportion do not have typical features. Approximately 50% of heterozygotes are cytogenetically negative and have a small CGG insert. These individuals usually do not demonstrate physical or cognitive involvement (Rousseau et al., 1991). With more significant CGG amplification (i.e., >200 repeats) women often demonstrate minimal physical involvement but may or may not

Table 1
Physical Features of Fragile X Males
Adapted from Hagerman and Brunschwig, 1991

Features	All Patients (all ages)	Prepubertal (<13 yrs old)	Pubertal & Postpubertal (>13 yrs old)
Long ears	105/172 = 61%	62/94 = 66%	43/78 = 55%
Prominent ears	107/165 = 65%	72/91 = 79%	35/74 = 47%
High-arched palate	78/164 = 48%	32/90 = 36%	46/74 = 62%
Prominent jaw	21/81 = 26%	3/32 = 9%	18/49 = 37%
Pectus excavatum	58/147 = 39%	24/82 = 29%	34/65 = 52%
Long face	106/134 = 79%	49/65 = 75%	57/69 = 83%
Hyperextensible MP* joints	108/171 = 63%	79/95 = 83%	29/76 = 38%
Hand calluses	48/145 = 33%	19/77 = 25%	29/68 = 43%
Double-jointed thumbs	50/116 = 43%	32/64 = 50%	18/52 = 35%
Single palmar crease	40/148 = 27%	22/84 = 26%	18/64 = 28%
Flat feet	89/147 = 61%	58/80 = 73%	31/67 = 46%
Macroorchidism	114/170 = 67%	42/91 = 46%	72/79 = 91%

*MP = Metacarpal phalangeal

demonstrate neuropsychological deficits. Fryns (1986) found that only 14% of normal IQ heterozygous females had typical facial stigmata. In females who are positive in cytogenetic studies typical physical features are more common and are seen in over 50% (Fryns, 1986; Cronister et al., 1991). Prominent ears and extreme shyness can be seen in over 75% of cytogenetically positive fragile X girls (Hagerman et al., 1992). In addition, over 30% of fragile X positive girls have significant attention deficit hyperactivity disorder (ADHD).

Approximately 50% of normal IQ heterozygotes demonstrate significant depression, which is hypothesized to be related to the FMR-1 gene (Reiss et al., 1988; Hagerman & Sobesky, 1989). More significantly affected women experience frontal deficits on neuropsychological testing even when their IQ is within the normal range (Mazzocco et al., 1992a). Frontal problems cause planning and organizational difficulties, attention deficits and difficulties with flexibility in problem-solving strategies (Mazzocco et al., 1992b). The frontal deficits appear to be the cause of ADHD symptoms including impulsivity, but may also be related to mood lability, which is a concern of many heterozygotes (Sobesky et al., these proceedings, and Sobesky et al., 1992). Preliminary studies of the CGG repeat length have not correlated significantly with IQ in heterozygotes except to differentiate the group with a small insert only (Mandel et al., 1992; Rousseau et al, 1991; Staley et al., 1992). In those with a longer repeat, other variables such as X inactivation may be important. Further studies are necessary before we will understand the molecular-clinical correlations in the heterozygote.

The neuroanatomical changes resulting from the absence of the FMR-1 protein are beginning to be understood. Rudelli et al. (1985) and Wisniewski et al. (1991) reported changes in the dendritic spine patterns which were abnormally long and tortuous with a reduced synaptic contact area. Musumeci et al. (1988) reported a characteristic EEG abnormality involving temporal or central spikes which are seen in over 50% of fragile X patients, although seizures occur in just under 20% (Wisniewski et al., 1991). Musumeci reported a worse prognosis for seizures when the spikes occur outside of the rolandic region and a significant correlation between the spike wave frequency and the severity of hyperactivity (these proceedings). Reiss (1992) presented detailed and controlled MRI studies of fragile X patients which demonstrated a decrease in white matter in the right hemisphere and a larger hippocampus in fragile X compared to controls. This is consistent with neuropsychological studies which show visual spatial deficits and memory strengths (Baumgardener et al., these proceedings). Reiss et al. (1991) have previously reported the dramatic finding of a small posterior cerebellar vermis in fragile X males and females. At this conference, Reiss also reported preliminary evidence of a more rapid loss of grey matter in fragile X compared to controls. These studies require further confirmation.

An exciting new finding was presented by Berry-Kravis (these proceedings) from Chicago. She reported that cyclic AMP (cAMP) levels are lower in fragile X males compared to mentally retarded or autistic controls in a platelet assay (Berry-Kravis and Huttenlocher, 1992). Cyclic AMP is an important second messenger system which subsequently influences a variety of neurotransmitters, ion channels and transcription processes important for neuronal structure. This key finding may be linked to the dendritic structural changes reported in fragile X and may cause changes in neurotransmitter systems or ion channels that could be related to behavior problems such as disinhibition. Hopefully, research in this area will lead to improved pharmacological approaches in treatment.

THE BEHAVIORAL PHENOTYPE

A variety of nonspecific behavior problems are consistently seen in fragile X boys (Table 2). Several autistic-like features listed below have been regularly reported since Brown et al. (1982) first noted the association between fragile X and autism. Controlled studies by Reiss and Freund (1990) demonstrate a characteristic profile of autistic features in fragile X. Braden and Turk (these proceedings) show that hand-flapping, perseverative speech and strong reactions to change in the environment are significantly increased in fragile X boys compared to developmentally disabled controls. Cohen (these proceedings) chronicles the association of fragile X and autism and reports on additional data which support this association. He highlights the significant and consistent finding of poor eye contact which is more dramatic in fragile X than in nonfragile X autistic patients (Cohen et al., 1989 and Cohen et al., 1991). There are unique aspects to the language of fragile X patients which Sudhalter pinpoints (these proceedings) in well designed controlled studies. Semantic deficits related to the inability to block or inhibit high associate responses are characteristic of fragile X speech and appear to be related to disinhibition, ADHD symptoms and perhaps the frontal deficits described in neuropsychological testing (Baumgardner et al., these proceedings). In addition, perseveration is a significant deficit for fragile X boys compared to controls and can be exacerbated by forced eye contact or anxiety (Sudhalter et al., 1990 and these proceedings).

Table 2
Behavioral Features of Fragile X Males
Adapted from Hagerman and Brunschwig, 1991

Features	All Patients (All ages)	Prepubertal (<13 yrs old)	Pubertal & Postpubertal (> 13 yrs old)
Hand-flapping	130/156 = 83%	77/87 = 89%	53/69 = 77%
Hand-biting	102/169 = 60%	49/91 = 54%	53/78 = 68%
Tactile defensiveness	84/105 = 80%	43/57 = 75%	41/48 = 85%
Poor eye contact - by history	136/150 = 91%	70/82 = 85%	66/68 = 97%
Poor eye contact - by exam	127/143 = 89%	70/83 = 84%	57/60 = 95%
Hyperactivity	111/150 = 74%	71/88 = 81%	40/62 = 65%
Short attention span only	27/150 = 18%	13/88 = 15%	14/62 = 23%
Perseveration	107/116 = 92%	58/64 = 91%	49/52 = 94%
Imitation skills	57/74 = 77%	29/35 = 83%	28/39 = 72%
Tantrums	79/105 = 75%	49/65 = 75%	30/40 = 75%
Physical aggression	54/90 = 60%	23/41 = 56%	31/49 = 63%

These features appear to be related to the unusual sensory processing and disinhibition which is clinically apparent in affected fragile X patients. Their frequent frustration and overstimulation from the environment often lead to tantrums and outburst behavior which require intervention such as calming techniques and counseling (see Intervention section of these proceedings). Although tantrums and outburst behavior are not significantly different from other developmentally disabled controls (Braden; Turk; and Maes et al., these proceedings), attentional problems are a significant difficulty even in adult fragile X males (Maes et al., these proceedings). Alterman et al. (these proceedings) report on specific rehabilitation strategies to deal with the significant attentional deficits in fragile X adult clients.

TREATMENT

Although there is no cure for fragile X and gene therapy appears to be years ahead, there are a variety of interventions which can be helpful to the majority of children and adults with fragile X syndrome. Appropriate intervention requires several professionals including the speech and language pathologist, occupational and physical therapist, special education teacher, genetic counselor and psychologist or therapist. Several papers in these proceedings outline intervention strategies in each of these areas. The following discussion will be limited to the physician's role in treatment.

The medical follow up of fragile X children includes treatment of medical complications associated with the connective tissue abnormalities and psychopharmacology for the behavior problems and attention deficits. In infancy and preschool years normalization of vision and hearing are essential. Recurrent otitis media is a problem for over 60% of fragile X children and should be treated aggressively with the insertion of PE tubes to maintain normal hearing (Hagerman, 1991b). Strabismus is seen in over 30% of affected children and nystagmus and ptosis have also been reported (Maino and King, these proceedings). Referral to an ophthalmologist is essential whenever visual abnormalities are noted on routine examination. All fragile X children, however, should be seen by an optometrist or ophthalmologist by 4 to 5 years of age for a routine eye examination.

Goldson reported (these proceedings) the occasional occurrence of gastroesophageal reflux (GER) in fragile X infants. He hypothesized a relationship to the connective tissue abnormality which can cause a floppy or incompetent lower esophageal sphincter similar to the abnormalities in the mitral valve. In addition, GER may foster failure to thrive (FTT) in the fragile X infant in association with other difficulties, such as easy gagging and frequent regurgitation (Goldson and Hagerman, 1992). Goldson's report in these proceedings outlines the medical follow up for these and other problems common to fragile X children.

The physician should take an active role in coordinating the genetic workup and subsequent counseling outlined in the Diagnostic section of these proceedings. In addition, the physician should coordinate the additional therapies such as speech and language therapy and occupational therapy which are essential for all fragile X children from the preschool period onward. Because of an unusual combination of learning strengths and weaknesses, fragile X children often have difficulties in school placement (Scharfenaker et al., 1991). They usually require mainstreaming in addition to special education help and individualized therapy which is further reviewed in the Intervention section of these proceedings.

Lastly, psychopharmacology can be helpful for fragile X children and adults (Hagerman, 1991b). The focus in childhood is on improvement of attentional problems, although mood lability and tantrums may also require treatment. Gualtieri (these proceedings) described the use of anticonvulsants, specifically carbamazepine or valproate, for treatment of behavior problems including aggression in fragile X patients. These medications can be used whether EEG abnormalities or seizures are present or not but they should be the medications of choice if seizures are present (Wisniewski et al., 1991).

The use of dopamine agonists is the mainstay of treatment for attentional problems and hyperactivity. Although the first line stimulants, methylphenidate (Ritalin), dextroamphetamine (Dexedrine) and pemoline (Cylert) are effective in the majority of fragile X school-age children with ADHD (Hagerman et al., 1988), side effects are common, such as irritability and poor appetite, particularly at higher doses. Therefore, alternatives are often used such as amantadine (see Gualtieri, these proceedings) and clonidine. The latter is an antihypertensive medication that has been helpful in treating tics and ADHD in Tourette's syndrome and hyperarousal in ADHD and in post-traumatic stress disorder (Hunt et al., 1990). Clonidine has been used successfully in fragile X patients as young as 4 years for treatment of ADHD symptoms and hyperarousal. The main side effect is sedation, so a very low dose is used initially, particularly in the young patient, i.e., 1/4 or 1/8 tablet (each tablet is 0.1 mg) twice a day. The sedation improves after two to three weeks and the dose can be increased gradually as tolerated to approximately 0.15

mg to 0.3 mg/day in the school-aged child. It is important to follow cardiovascular parameters regularly, particularly blood pressure, and the dose should be decreased if dizziness or nausea occur.

Turk (these proceedings) presents details of his folic acid study and evidence that folate impacts both the dopamine system and the serotonin system. This is consistent with the clinical observations that folate works in a similar fashion to a weak stimulant medication (Hagerman, 1991b). It can be used in infancy and early childhood, unlike many of the other medications described here.

In adolescence and adulthood ADHD symptoms may improve but mood lability and temper outbursts are a problem for many fragile X affected individuals. Psychotherapy can be beneficial as described in reports by Brown and Baum in these proceedings and Brown et al., (1991). However, medication can be additionally beneficial and the focus in adolescence and adulthood is on serotonergic agents. Lithium has been used successfully in the past (Hagerman, 1991b) but it requires frequent monitoring of blood levels and avoidance of renal toxicity. Most recently, fluoxetine (Prozac), a serotonin re-uptake blocker, has been beneficial in open trials for the treatment of episodic dyscontrol or aggression. (See Preskorn these proceedings for a discussion of serotonin.) It appears to improve mood lability, calm the hyperarousal and decrease outbursts in many fragile X patients. Fluoxetine raises cAMP levels so it may counteract some of the effects of absent FMR-1 protein. Fluoxetine has been beneficial to heterozygotes who suffer from depression or mood lability. In a survey of 12 heterozygotes who had taken fluoxetine for these problems, 10 reported great benefit without significant side effects. The doses ranged from 20 to 40 mg. of fluoxetine/day. An additional serotonergic agent that has been anecdotally helpful for anxiety in fragile X males and females is buspirone. Since buspirone does not have the side effects of sedation and addiction which are seen with benzodiazepines, it may be the drug of first choice for treatment of anxiety symptoms in fragile X.

Controlled studies are needed to clarify the efficacy of these serotonergic medications in fragile X. They appear to be beneficial to control aggression and they should be used instead of antipsychotic major tranquilizers for this purpose. However, psychotic ideation is not uncommon in fragile X and requires careful evaluation and the judicious use of antipsychotic medication in some circumstances (see report by Levitas in these proceedings).

In conclusion, a variety of psychopharmacological agents are beneficial to fragile X children and adults. Their use requires a detailed assessment of the patient and the utilization of other helpful therapies in addition to medication. Finding the right medication may also be a trial and error process which necessitates optimal communication between the parents and the physician.

ACKNOWLEDGEMENTS

I gratefully acknowledge support from Joslins for the Fragile X Project at The Children's Hospital in Denver and support from NIMH grant #45916 for our research.

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THE MEDICAL MANAGEMENT AND FOLLOW-UP OF FRAGILE X CHILDREN

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INTRODUCTION

Children with fragile X (fra X) syndrome have a unique constellation of physical, psychological, cognitive and behavioral characteristics. However, they are also children who are subject to many of the problems that occur in otherwise normal children. None of the problems discussed below are unique to fra X children, but these children are at greater risk for these difficulties. Therefore, the fra X child needs to be closely monitored and diagnosed early, rigorously treated and closely followed.

RECURRENT OTITIS MEDIA (middle ear infections)

Otitis media is probably one of the most common minor acute illnesses in childhood. However, it is known to occur more frequently among fra X children than in the general population (Hagerman et al., 1987). It is imperative for parents and clinicians to identify the child with an acute ear infection and to treat the illness vigorously and then follow the child closely to be sure the acute process has resolved and to avoid chronic problems. The acute disease is treated with antibiotics while the chronic or recurrent illness may require treatment with steroids, prophylactic antibiotics or the placement of PE tubes. If chronic or recurrent ear infections develop, it is important to consult an otolaryngologist (ENT) to identify treatment options.

Associated with chronic ear infections is the increased risk of the child having a hearing loss when infections persist or fluid accumulates in the middle ear. It is critical to monitor this in order to avoid compromising the child's language development. It is known that fra X children have language difficulties and it is imperative these difficulties not be made more complicated by hearing loss.

OPHTHALMOLOGIC DISORDERS

As we learned in another session (Maino & King, these proceedings) at the conference, fra X children have a high incidence of refractive errors (that is they are either far-sighted or near-sighted) and also have an increased incidence of strabismus (lazy eye). As with children in the general population, it is essential to identify and treat these disorders early as they do not go away. This can be done, if necessary, in the first year of life and is particularly important for children with strabismus to preserve their depth perception. A referral to a pediatric ophthalmologist is essential to receive appropriate treatment. Once treatment is instituted it is important to monitor vision as conditions can change, and changes in treatment may be required. It should be borne in mind that these are treatable disorders and no child should be compromised because of a correctable visual difficulty (Maino et al., 1991).

FEEDING DISORDERS

These are very common problems for children with fra X syndrome (Goldson and Hagerman, 1992). The symptoms may include vomiting, food refusal, difficulty chewing and swallowing, gagging and food hoarding in the mouth, all of which may result in poor weight gain and are stressful for parents as they attempt to feed their child. Most of the time, these problems improve with age and minor modifications in the feeding regimen. However, when symptoms persist or the child is not thriving, a more detailed evaluation is required. Oral-motor dysfunction should be considered as well as gastroesophageal reflux (GER). The evaluation should include an oral-motor assessment, a complete blood count to rule out anemia, and an evaluation of the stool to determine whether or not blood is present. If vomiting, gagging or coughing with feeds are present, an evaluation of the upper gastrointestinal tract should be considered. If GER is likely, a study to look for the presence of gastric acid in the esophagus (pH study) can be performed to document the presence of GER and confirm the diagnosis. Consultation with a pediatric gastroenterologist can be helpful. Under some circumstances, the gastroenterologist may recommend endoscopy to determine whether esophagitis (inflammation of the esophagus) is present. Occult or asymptomatic esophagitis can be the cause of anemia and the source of chronic pain for these children and can lead to irritability, vomiting and disorganized behavior.

If the diagnosis of GER is made, there are a number of treatment options, medical and surgical. The medical management includes elevation of the bed at night and more frequent but smaller feeds, particularly for the very young child. Drugs used to treat GER include bethanechol (0.1 mg/kg/dose, four times a day given 15 minutes before a meal and before sleep) and metaclopramide (0.1 mg/kg/dose four times a day). Bethanechol is an anticholinergic drug that increases the esophageal "sphincter" tone and may decrease gastric emptying time while metaclopramide decreases gastric emptying time. Both of these drugs serve to decrease the amount of reflux the child has and so improve vomiting and esophagitis. If there is a concern about esophagitis, cimetadine (25-30 mg/kg/day in four divided doses) or ranitidine (7-10 mg/kg/day in two divided doses), which inhibit gastric acid secretion, can be used to prevent or ameliorate esophagitis.

If medical management is ineffective in treating GER, surgical intervention is another option. The operation performed is called a fundoplication. The upper part of the stomach is wrapped around the bottom of the esophagus so creating a sphincter which prevents the reflux of gastric contents into the esophagus thus alleviating esophageal irritation and vomiting. On occasion, both medical and surgical approaches may be necessary to control GER and its sequelae.

Following the diagnosis and institution of treatment, it is necessary to provide close follow-up. Food intake and weight gain as well as hemoglobin levels should be closely monitored.

SEIZURES (EPILEPSY)

Seizures have been identified in approximately 20% of fra X children (Wisniewski et al., 1991; Musumeci et al., 1988). During this meeting, abnormal electroencephalograms (EEGs) have been shown to be associated with seizures although there can frequently be an abnormal EEG without clinical seizures (Musumeci et al., these proceedings). One of the questions raised has been whether abnormal EEGs may be associated with sleep disorders often seen in fra X children. Can one ameliorate the sleep disorder without overt seizures in the presence of an abnormal EEG with anticonvulsants? This has not been clearly defined and is definitely an area that warrants further research. In any event, when seizures are identified, they obviously should be treated and the efficacy of that treatment closely monitored.

ORTHOPEDIC PROBLEMS

Orthopedic difficulties, although not as serious as the other problems noted above, are still significant for children with the fra X syndrome (Davids et al., 1990). The most common problems include flat feet (pes planus), excessive joint laxity, and scoliosis. Club feet and patellar dislocations have also been reported. The flat feet and joint laxity occur in over 50% of boys with the syndrome and may be helpful in considering the diagnosis of fra X syndrome. When patients first present, these problems rarely need intervention unless trauma or a joint dislocation occurs. Many fra X boys are hypotonic and have a clumsy gait and orthopedic intervention, such as the use of orthotics or orthopedic shoes is occasionally helpful in facilitating a more normal gait.

The purpose of this brief discussion is to alert both parents and clinicians to common problems such as otitis media, eye problems, eating disorders, seizures and orthopedic problems that occur frequently among fra X children. It is essential these problems be diagnosed early, vigorously treated and closely monitored.

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PHYSICAL CHARACTERISTICS OF YOUNG BOYS WITH FRAGILE X SYNDROME

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INTRODUCTION

Numerous articles have summarized physical manifestations of males with fragile X syndrome (Butler et al., 1991a; Butler et al., 1992; Hagerman, 1987; Meryash et al., 1984; Partington, 1984; Simko et al., 1989). In the adult male, commonly described physical characteristics include a broad forehead, an elongated face, large and prominent ears, and enlarged testicles (Laing et al., 1991; Hagerman et al., 1991; Butler et al., 1991b). These features do not occur in all males with fragile X, and some males might not be recognized by physical appearance alone (Hagerman et al., 1988). Black males with fragile X have been reported to have fewer abnormal facial features of fragile X than white males, but information on minority populations has been more limited than on white males (Schwartz et al., 1988; Stevenson and Prouty, 1988).

This report describes physical manifestations of prepubertal boys with fragile X who presented to our fragile X project between late 1988 and early 1992. Their physical characteristics were compared to a control group of Caucasian boys who were tested for fragile X. Some physical features such as finger tip patterns or ocular measurements were not studied because these boys were often very difficult to examine. Rather, we used a rating scale we developed, which included a brief medical history and physical examination. One reason for attempting this study was to determine the feasibility of screening school-aged boys for fragile X syndrome by assessing physical manifestations.

METHODS

A fragile X rating scale was developed after examining approximately 20 males with fragile X syndrome and after having reviewed the literature on physical characteristics of males with fragile X. Questions about the boy's medical history and family history were filled out by the parents prior to his appointment. This information was reviewed by the author with the parents. All boys in this report were examined by the author.

Head circumferences and ear lengths were measured using a plastic measuring tape. Heights and weights were obtained on a standard upright scale with an attached measuring stick. Prader orchiometer beads were used to estimate testicular volume.

Physical Characteristics of Young Boys

Some physical characteristics were determined subjectively. Hyperextensible joints referred to the metacarpophalangeal joints and were recorded as ++ when there was approximately 90 degrees or more of extension. Greater than 65 to 70 degrees of extension was recorded as +. Less than that was recorded as -. Brisk knee and ankle reflexes were recorded as +, normal or low reflexes were recorded as -. Many boys had previous developmental assessments and when the examiner had difficulty assessing muscle tone, previous occupational therapy or physical therapy reports were consulted. The hallucal crease referred to a crease more than one centimeter in length between the first and second toes.

Many boys were followed by local health providers and, in general, we did not refer them for specific medical consultations such as cardiology, otolaryngology, or neurology unless medically indicated or unless the parents requested to use our services rather than local services. This limited some of the information that was available to us.

This author initially planned to study only those boys who presented to our Child Development Unit to be tested for fragile X; however, most boys with fragile X who visited our clinic were diagnosed by local physicians or in genetics clinic. Only four of the boys with fragile X were examined prior to diagnosis using the rating scale. On the other hand, 20 out of 29 controls were examined before they were karyotyped or before DNA studies were done.

RESULTS

A summary of the findings are listed.

Medical History:	**Fragile X** (N=29)	**Controls** (N=29)
Weighed > 8 lbs at Birth	21	15
Hernias	3 inguinal 1 umbilical	2 inguinal
Eye Problems	13*	5
Cleft Lip/Palate	1	0
Curvature of the Spine	1	0
More than 5 Ear Infections	22	17
Seizures	3	4
Allergies Except for Antibiotics	8	10
History of Mental Retardation	27	18
Any Positive Family History of Mental Retardation	22	12

Physical Examination:

See figures for comparisons of head circumferences, heights, weights, and testicular sizes.

Positive Findings	**Fragile X**	**Controls**
Head Circumference > 50th Percentile	22/29	21/29
Broad Forehead	22/29	20/29
Elongated Face	24/29	14/29
Ocular Abnormalities	9/29*	4/29
Poor Eye Contact/Gaze Avoidance	24/29	16/29
Inability to Close Eyes on Request	2/18	0/23
Ears Longer than 75th Percentile	24/29*	12/29
Simply Formed Helices	9/29+	3/29
Prominent Ears	20/29	11/29
Highly Arched Palate	27/29	18/29
Pectus Excavatum	13/29*	6/29
Mitral Click	1/29	0/29
Curvature of the Spine	2/29	0/29
Testicles $\geq$ 4 cm^3	7/29+	0/29
Hyperextensible Joints (++)	23/29	12/29
(+)	6/29	8/29
Curved 5th Finger	17/29	15/29
Abnl Palmar Creases/Distal Axial Triradii	7/29	4/29
Calluses on Hands from Biting	9/29+	1/29
Soft Skin over Dorsum of Hand	28/28	21/29
Flat Feet	21/29	19/29
Hallucal Crease	22/28*	8/29
Hypotonia	21/29	14/29
Brisk Knee and Ankle Reflexes	21/29	11/29
Adverse Response to Touch on Skin	19/29*	7/29
Difficulty Touching Lips with Tongue	19/22+	6/21
Difficulty Moving Protruded Tongue from Side to Side	14/21+	3/21
Difficulty Saying "Puh-tuh-kuh"	16/21*	7/18
Difficulty Saying "Linoleum"	18/21	14/19

(* occurred $\geq$ twice as often in boys with fragile X;
+ occurred $\geq$ more than three times as often in boys with fragile X)

DISCUSSION

The boys with fragile X displayed many abnormal physical characteristics compared to controls. These included an elongated face, long ears, hand calluses, hyperextensible joints and oral-motor incoordination. All of these physical manifestations should be useful for screening boys for fragile X. Some findings, however, were subtle and it was often difficult to determine how best to describe them. For example, some of the boys, who were classified as having pectus excavatum, had minimal findings that might not be noted during a routine physical examination. Hypotonia was very difficult to assess in some of the older boys, who looked fairly good on a routine physical, but who were described as being hypotonic by an occupational therapist. While

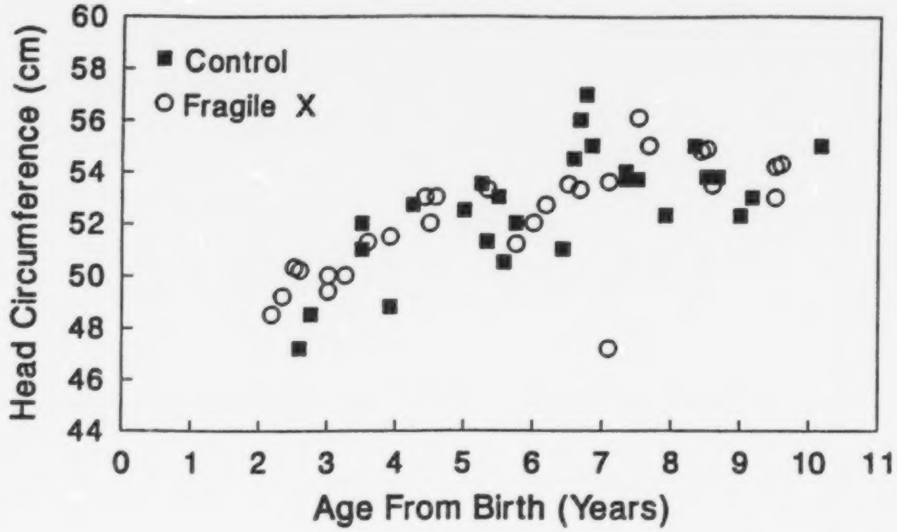

Figure 1: Head circumferences of boys with fragile X compared to controls.

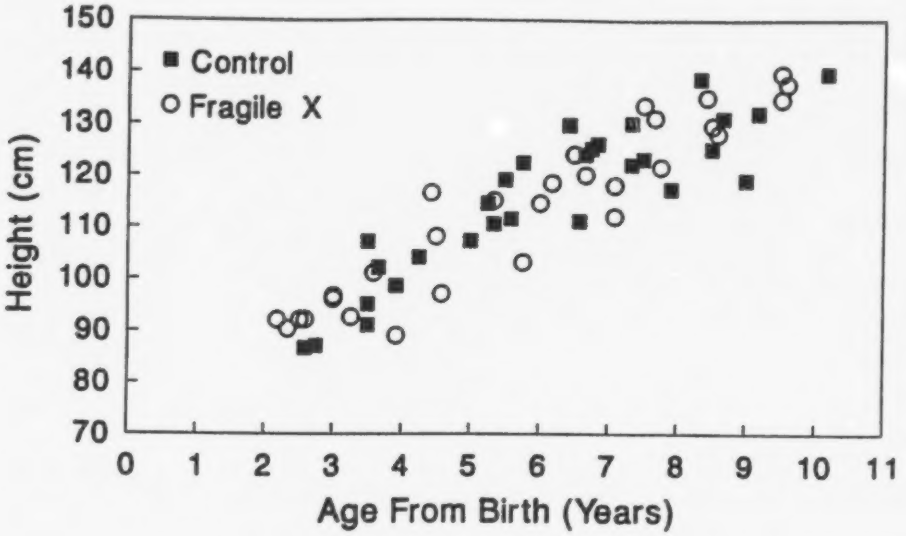

Figure 2: Heights of boys with fragile X compared to controls.

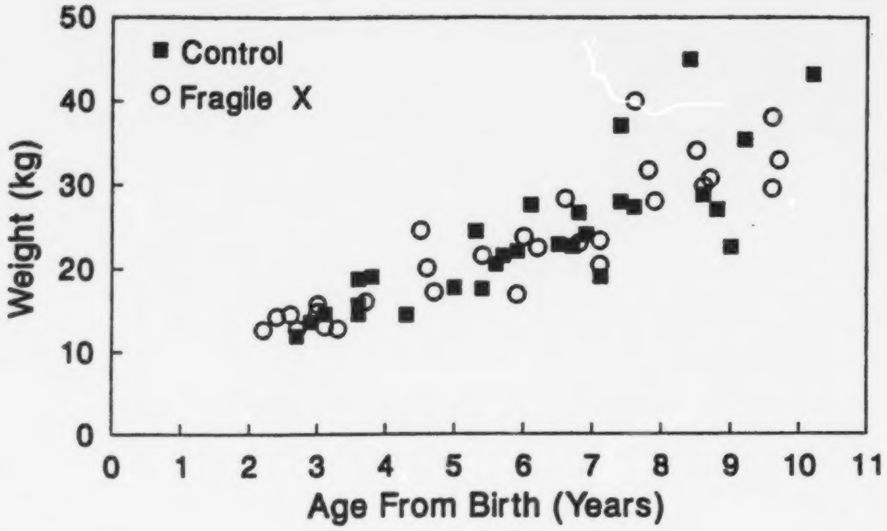

Figure 3: Weights of boys with fragile X compared to controls

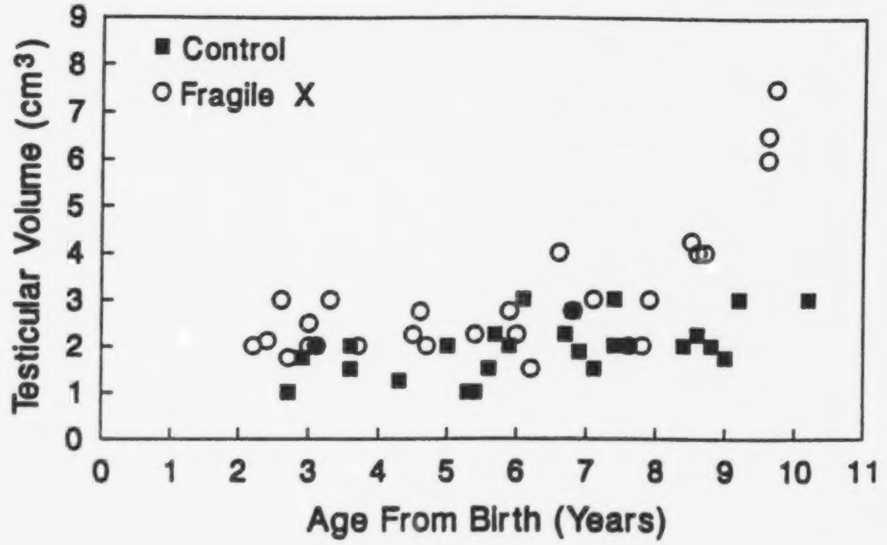

Figure 4: Testicular volume of boys with fragile X compared to controls

mitral valve prolapse may be more common than reported in this study (Hagerman and Synhorst, 1984), mitral valve clicks are rarely appreciated by this examiner during a regular physical examination on young boys. The boys were often too cognitively impaired to request appropriate maneuvers. The high percentage of boys with ophthalmologic problems was unexpected and findings included strabismus, myopia, hyperopia, nystagmus (one child) and abnormal optic disks (one child). Testicular enlargement did not appear to be a useful indicator of fragile X in the younger boys (Figure 4). Some boys were unable to cooperate for the oral-motor assessment, and no answers were recorded for several boys.

Only three boys with fragile X syndrome had clear histories of seizures and all were well-controlled by medication. One boy had possible seizures (staring spells) treated with Tegretol for a short time. When medication was discontinued, no further episodes occurred. Two other children had staring spells that raised concerns about seizures. One of these boys had an abnormal EEG with diffuse background slowing and right occipital epileptiform discharges. He was taking Tegretol and Dexedrine for behavior and, possibly, seizure control.

We did not explore the issue of allergies in depth. Most of the parents, who described a positive history of allergies, had not had their boy tested. Only one boy with fragile X is actively followed in our allergy clinic.

Only one black child with fragile X was examined during the years we collected this information. His original karyotype (prior to the isolation of the FMR-1 gene) was negative for fragile X. Because of a very strong family history for X-linked mental retardation and physical characteristics consistent with fragile X, DNA studies were done and were positive. Because we serve a large population of black children (almost 50%) in our other clinics, it concerns us we have not diagnosed more black children. We do not know if there is a reduced incidence of fragile X in this population or if the diagnosis is not being made.

We did not expect so many of the young boys with fragile X to be classified as mentally retarded. Perhaps in a larger series, more boys might test above the mentally retarded range. Only one boy in our series tested in the borderline range. The other boy was two years old, had no expressive language, but had relatively good receptive language, fine motor, and adaptive skills.

Two of the controls had positive family histories for fragile X. One boy was receiving special education services for a speech and language delay, but his DNA studies were normal. The other boy was normal on developmental testing and did not have fragile X on DNA studies. Several of the other controls, with and without mental retardation, had relatives with autism or some other type of developmental disability. One autistic boy had a mentally retarded sister.

Five boys had 1 to 2% fragility on karyotype. Four out of five were reclassified as being negative for fragile X once DNA studies became available. These findings suggest over 10% of individuals who are currently classified as having fragile X based on low fragility may be misclassified and should be restudied. Only one remained positive for fragile X on DNA studies. This was consistent with their clinical appearances. One boy, who was negative for fragile X, was positive clinically and, subsequently, on DNA studies (Brooks et al., 1991). While none of the other boys, who were negative on karyotype, appeared to have fragile X, several had worrisome behaviors and clinical features compatible with fragile X. They will be brought back to our clinic for fragile X DNA studies. The clinical assessment can be useful in determining who needs to be restudied.

None of the controls who were tested by karyotype had any other chromosomal abnormality. Four boys had unusual physical characteristics not usually associated with fragile X syndrome. One boy was thought to have both fragile X syndrome and Pfieffer syndrome. One boy had microcephaly. Another had hydrocephalus and a shunt. One boy had nystagmus, epicanthal folds and pulmonary valve stenosis. These four boys would be difficult to detect by physical examination alone, yet each had some manifestations of fragile X, particularly hyperextensible joints.

SUMMARY

We hoped this study would provide us with a clear phenotype of boys with fragile X compared to other boys with developmental delays. Although no single physical characteristic always correlated with fragile X, some features suggested fragile X and should be used for screening large populations of boys. Elongated faces, large or simply shaped helices, hand calluses and oral-motor incoordination were among the characteristics seen more often in boys with fragile X. Large testicles were suggestive of fragile X in the older boys. Visual problems were more common in the boys with fragile X than controls. Because physical characteristics are not diagnostic, all young boys with significant developmental delays should be studied for fragile X syndrome.

ACKNOWLEDGMENTS

The fragile X outreach project is indebted to several funding agencies including the March of Dimes Birth Defects Foundation (Social and Behavioral Sciences Grant #12-195), the North Carolina Civitans, the Duke Telethon Committee, the North Carolina Knights of Columbus, and the North Carolina Council on Developmental Disabilities.

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ANTHROPOMETRIC AND CRANIOFACIAL PATTERNS IN MENTALLY RETARDED MALES WITH AND WITHOUT THE FRAGILE X SYNDROME

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ABSTRACT

Anthropometric and craniofacial profile patterns indicating the percent difference from the overall mean were developed on 34 physical parameters with 31 white mentally retarded males with the fragile X syndrome matched for age with 31 white mentally retarded males without the fragile X syndrome. The fragile X syndrome males consistently showed larger dimensions for all anthropometric variables with significant differences for height, sitting height, arm span, hand length, middle finger length, hand breadth, foot length, foot breadth, and testicular volume. A craniofacial pattern did emerge between the two groups of mentally retarded males, but with overlap of several variables. Significant differences were noted for head circumference, head breadth, lower face height, bizygomatic diameter, inner canthal distance, ear length and ear width with the fragile X syndrome males having larger head dimensions (head circumference, head breadth, head length, face height and lower face height), but smaller measurements for minimal frontal diameter, bizygomatic diameter, bigonial diameter, and inner canthal distance. In a combined anthropometric and craniofacial profile of 19 variables comparing 26 white fragile X syndrome males [13 with high expression (>30%) and 13 with low expression (<30%), but matched for age], a relatively flat profile was observed with no significant differences for any of the variables. Thus, there appears to be no physical differences detectable with this methodology between fragile X males with high or low expression. Our research illustrates the use of anthropometry in identifying differences between mentally retarded males with or without the fragile X syndrome and offers a comprehensive approach for screening males for the fragile X syndrome and selecting those individuals for cytogenetic and/or molecular genetic tests.

INTRODUCTION

Anthropometric methods have been applied increasingly in the clinical evaluation and diagnosis of individuals with genetic disorders and other health problems (Dequeker et al., 1983, Meaney and Farrer, 1986, Meaney and Butler, 1989, Butler et al., 1991a). One of these conditions is the fragile X (fra X) or Martin-Bell syndrome, the most common familial cause of male mental retardation. This syndrome is characterized by mental retardation, macroorchidism, large or prominent ears, a long narrow face, hyperflexibility, and a characteristic chromosome fragile site at Xq27.3 (Turner et al., 1986, Hagerman, 1987, Butler, 1988). The incidence of the fragile X syndrome is approximately one in 1,000 males (Herbst and Miller, 1980, Turner et al., 1986, Webb et al., 1986). Unstable DNA sequences representing large increases in the number of CGG repeats have recently been located at the fragile site and characterization of the gene (FMR-1) implicated in the fra X is underway at several medical centers (Yu et al., 1991, Oberle et al., 1991, Rousseau et al., 1991, Sutherland et al., 1991, Verkerk et al., 1991, Pergolizzi et al., 1992). Alterations of the FMR-1 gene, detectable with several DNA probes with Southern blotting analysis and more recently with PCR methods, are gaining acceptance for diagnostic and clinical applications (Pieretti et al., 1991, Pergolizzi et al., 1992).

Due to phenotypic variability, early recognition of fra X individuals can be difficult. Hagerman et al. (1988) reported that the presence of large ears, macroorchidism and hand calluses in mentally retarded males would yield positive fragile X chromosome results in 50% of these patients. Recently, Butler et al (1991a, 1991b) and Hagerman et al. (1991) reported that certain physical characteristics (e.g., ear width, testicular volume, bizygomatic diameter, head breadth, plantar crease, hyperflexibility) could be used to correctly classify the fra X syndrome in greater than 90% of mentally retarded males. Butler et al. (1992) also reported anthropometric standards for clinical use for weight, height, head circumference, ear length and testicular volume for fra X males. Until recently, only a few studies were reported on the linear growth patterns in fra X individuals (Partington, 1984, Meryash et al., 1984, Sutherland and Hecht, 1985; Loesch et al., 1988; Butler et al., 1988a, Butler et al., 1988b, Saul, 1988). Our current research focuses on the development of anthropometric and craniofacial patterning profiles of mentally retarded males with and without the fra X syndrome through a comprehensive assessment of measurements at several body sites. The effects of fra X chromosome expression on anthropometric variables of affected males will also be presented.

MATERIALS AND METHODS

A cytogenetic and clinical survey of 236 mentally retarded males (39 with and 197 without the fra X syndrome) was undertaken recently including a comprehensive assessment of anthropometric variables, and significant differences were reported for 18 measurements (weight, height, 7 linear, 2 breadth, 5 craniofacial, 1 skinfold and testicular volume) (Butler et al., 1991a, Butler and Singh, 1992). A subset of these individuals were used in the current study to compare anthropometric and craniofacial profile patterns between mentally retarded males with and without the fra X syndrome and, in addition, affected males with either high or low fra X chromosome expression. Specifically, 31 mentally retarded white males diagnosed with the fra X syndrome with an average age of 29.5 years and an average range of 3.7 years to 75.6 years were matched for age (within 1 year before 18 years of age and within 2 years if greater than 18 years) with 31 white mentally retarded males without a diagnosis or cause of their mental retardation with an average age of 29.5 years and an age range of 3.8 years to 74.7 years. Thirteen white fra X males with high expression (>30% with an average of 43% and range of 32% in 58% in cells grown routinely in folate deficient culture conditions in medium 199) were matched for age (within 1 year before 18 years of age and within 2 years if greater than 18 years) with 13 white fra X males with low fra X chromosome expression (<30% with an average of 18% and a range of 3% to 27% in cells grown routinely in folate deficient culture conditions) (Butler et al., 1990).

All measurements and physical examinations were made by one of the authors (M.G.B., M.S.W. or W.R.B.) according to standard techniques as presented by Weiner and Lourie (1969) and Farkas (1981). The anthropometric evaluation for this study consisted of 34 measurements including 19 anthropometric variables (weight, height, sitting height, arm span, shoulder-elbow length, elbow-wrist length, elbow-fingertip length, hand length, middle finger length, palm length, hand breadth, wrist breadth, knee-buttock length, foot length, foot breadth, ankle breadth, tricep skinfold, subscapular skinfold and testicular volume) and 15 craniofacial variables (head circumference, head breadth, head length, cephalic index (head breadth/head length X 100), face height (gnathion-trichion), lower face height (gnathion-nasion), minimal frontal diameter, bizygomatic diameter, bigonial diameter, inner canthal distance, outer canthal distance, nose length, nose breadth, ear length and ear width). Skinfold measurements were recorded to the nearest 0.5mm with a Lange skinfold caliper. Arm span and head circumference were recorded to the nearest mm with a steel tape. Weight was measured with a balanced-beam scale, height and linear values were measured with an anthropometer or sliding calipers, breadth measurements were obtained with a spreading caliper, and testicular volume was estimated by the use of a Prader orchidometer (if greater than 25mL, the length and width were measured with a sliding caliper and the volume was calculated by the use of the equation $\pi/6 \times L \times W^2$). Matched t tests were used to identify significant differences for each variable between age-matched mentally retarded males with or without the fra X and between fra X males with high fra X chromosome expression (>30%) or low expression (<30%).

RESULTS

Figure 1 displays an anthropometric profile indicating the percent difference from the overall mean comparing the 31 white mentally retarded males with the fra X syndrome matched for age with 31 white mentally retarded males without the fra X syndrome. The average age ± standard deviation for the fra X males was 29.5 ± 16.0 years (age range was 3.7 to 75.6 years) with an average ± standard deviation for fra X chromosome expression of 28% ± 14% in peripheral blood cells grown routinely in folate deficient culture conditions (Butler et al., 1990)

The average age ± standard deviation for the 31 non-fra X males was 29.5 ± 15.9 years (age range of 3.8 to 74.7 years). an anthropometric pattern does emerge between the two groups of mentally retarded males with or without the fra X syndrome and with the fra X males consistently showing larger dimensions for all variables with significant differences (matched t-test, $p < 0.05$) between the two groups for height, sitting height, arm span, hand length, middle finger length, hand breadth, foot length, foot breadth and testicular volume. The greatest differences were found for foot length, foot breadth and testicular volume. Table 1 shows the average ± standard deviation and t-test value for each anthropometric and craniofacial variable for the two groups of 31 matched mentally retarded males with or without the fra X. Table 2 shows summary anthropometric and craniofacial variables with significant differences between fragile X and non-fragile X mentally retarded males.

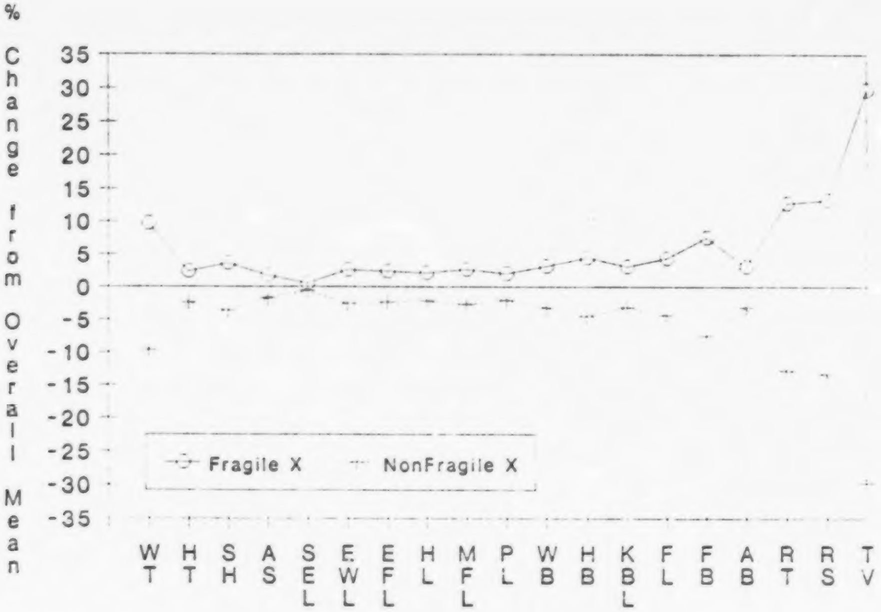

Figure 1. Anthropometric profile indicating percent difference from overall mean for 31 white mentally retarded males with the fragile X syndrome matched for age with 31 white mentally retarded males with no known cause of their retardation. WT = weight. HT = height. SH = sitting height. AS = arm span. SEL = shoulder-elbow length. EWL = elbow-wrist length. EFL = elbow-fingertip length. HL = hand length. MFL = middle finger length. PL = palm length. WB = wrist breadth. HB = hand breadth. KBL = knee-buttock length. FL = foot length. FB = foot breadth. AB = ankle breadth. RT = right triceps skinfold. RS = right subscapular skinfold. TV = testicular volume. 1 = $p < 0.05$ (two-tailed matched t-test). 2 = $p < 0.01$ (two-tailed matched t-test). 3 = $p < 0.001$ (two tailed matched t-test).

Table 1
Anthropometric and Craniofacial Patterning Data from 31 White Fragile X Males Matched for Age with 31 Mentally Retarded White Males Without a Cause of Their Retardation

Variables	Fragile X Males (Mean ± SD)	Non-fragile X Males (Mean ± SD)	Matched T Test Value
Weight (kg)	54.5 ± 25.2	44.9 ± 23.0	1.57
Height (cm)	162.8 ± 20.0	155.2 ± 21.8	3.18^b
Sitting height (cm)	83.0 ± 9.1	77.2 ± 10.7	2.82^a
Arm span (cm)	161.7 ± 21.1	156.2 ± 21.5	2.07^a
Head circumference (cm)	55.9 ± 2.9	53.9 ± 2.9	2.95^b
Head breadth (cm)	14.9 ± 0.8	14.3 ± 0.8	3.19^b
Head length (cm)	19.2 ± 1.2	18.6 ± 1.1	2.04
Face height (cm)	18.2 ± 1.5	17.5 ± 2.2	1.34
Lower face height (cm)	11.8 ± 1.4	11.1 ± 1.4	2.42^a
Minimal frontal diameter (cm)	10.5 ± 0.6	10.7 ± 0.7	0.89
Bizygomatic diameter (cm)	12.5 ± 1.1	13.0 ± 1.1	2.62^a
Bigonial diameter (cm)	10.3 ± 1.0	10.5 ± 1.0	1.02
Inner canthal distance (cm)	2.8 ± 0.4	3.1 ± 0.3	2.35^a
Outer canthal distance (cm)	8.7 ± 0.8	8.4 ± 0.7	1.68
Nose length (cm)	4.5 ± 0.7	4.5 ± 0.9	0.40
Nose breadth (cm)	3.5 ± 0.3	3.5 ± 0.5	0.00
Ear length (cm)	7.0 ± 0.7	6.6 ± 0.8	2.38^a
Ear width (cm)	4.3 ± 0.5	3.6 ± 0.4	7.08^c
Knee-buttock length (cm)	51.8 ± 9.8	48.7 ± 10.0	1.77
Shoulder-elbow length (cm)	32.6 ± 5.8	32.2 ± 6.8	0.30
Wrist breadth (cm)	5.2 ± 0.7	4.9 ± 0.8	1.86
Hand breadth (cm)	7.8 ± 1.1	7.1 ± 1.1	3.70^b
Middle finger length (cm)	7.6 ± 0.9	7.2 ± 0.9	3.23^b
Hand length (cm)	17.6 ± 2.0	16.8 ± 2.2	2.37^a
Palm length (cm)	10.1 ± 1.2	9.7 ± 1.6	1.48
Elbow-finger tip length (cm)	41.3 ± 7.0	39.4 ± 7.9	1.80
Elbow-wrist length (cm)	25.8 ± 5.8	24.5 ± 5.0	0.91
Ankle breadth (cm)	6.8 ± 0.7	6.4 ± 1.0	1.98
Foot length (cm)	24.6 ± 2.8	22.6 ± 2.7	4.61^c
Foot breadth (cm)	9.2 ± 1.5	7.9 ± 1.1	4.65^c
Triceps skinfold (cm)	13.6 ± 7.0	10.5 ± 4.5	1.95
Subscapular skinfold (cm)	13.9 ± 6.9	10.6 ± 5.8	1.11
Testicular volume (ml)	43.0 ± 27.6	23.3 ± 16.3	3.66^c
Cephalic index	77.5 ± 3.0	76.6 ± 3.5	1.03

a = $p<0.05$ (two-tailed)
b = $p<0.01$ (two-tailed)
c = $p<0.001$ (two-tailed)

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Table 2
Summary of Significant Anthropometric and Craniofacial Variables in Fragile X and Non-fragile X Males

Variables Significantly Larger in Fragile X Males (Significance Level)		Variables Significantly Larger in Non-Fragile X Males (Significance Level)
Height	($p< 0.01$)	
Sitting height	($p< 0.05$)	Bizygomatic diameter ($p<0.05$)
Arm span	($p<0.05$)	Inner canthal distance ($p<0.05$)
Head circumference	($p<0.01$)	
Head breadth	($p< 0.01$)	
Lower face height	($p<0.05$)	
Ear length	($p< 0.05$)	
Ear width	($p<0.001$)	
Hand breadth	($p<0.01$)	
Middle finger length	($p<0.01$)	
Hand length	($p<0.05$)	
Foot length	($p<0.001$)	
Foot breadth	($p<0.001$)	
Testicular volume	($p<0.001$)	

Figure 2 displays a craniofacial profile indicating the percent difference from the overall mean comparing the 31 white mentally retarded males without the fra X syndrome. A craniofacial pattern does emerge, but with overlap of several variables between the two groups of males with significant differences (matched t-test: $p < 0.05$) for head circumference, head breadth, lower face height, bizygomatic diameter, inner canthal distance, ear length and ear width. The greatest difference was found for ear width. The fra X males have larger head dimensions including head circumference, head breadth, head length, face height and lower face height, but smaller measurements for minimal frontal diameter, bizygomatic diameter, bigonial diameter, and inner canthal distance. In addition, outer canthal distance, nose length, ear length and ear width were greater in the fra X males.

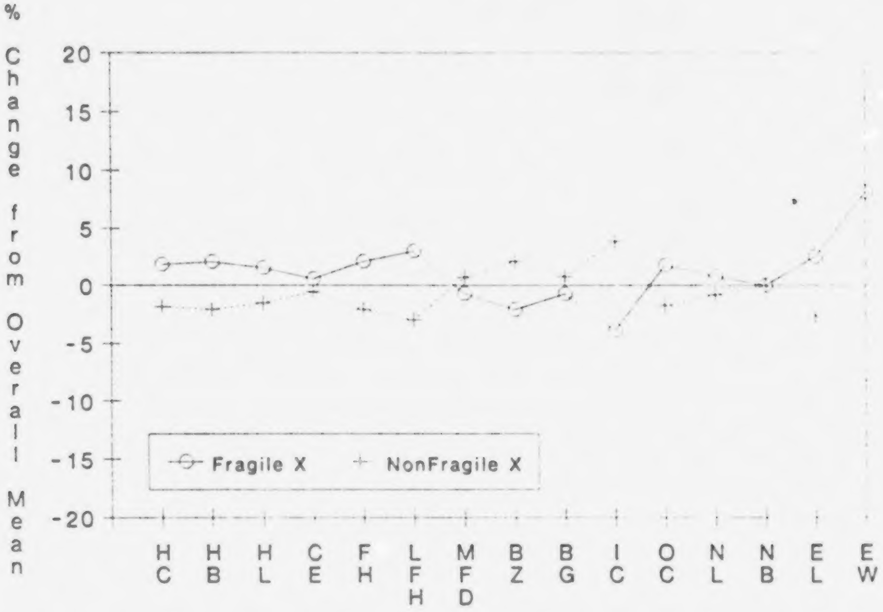

Figure 2. Craniofacial profile indicating percent difference from overall mean for 31 white mentally retarded males with the fragile X syndrome matched for age with 31 white mentally retarded males with no known cause of their retardation. HC = head circumference, HB = head breadth, HL = head length, CE = cephalic index, FH = face height, LFH = lower face height, MFD = minimal frontal diameter, BZ = bizygomatic diamter, BG = bigonial diameter, IC = inner cnathal distance, OC = outer canthal distance, NL = nose length, NB = nose breadth, EL = ear length, EW = ear width. 1 = $p < 0.05$ (two-tailed matched t-test), 2 = $p < 0.01$ (two-tailed matched t-test), 3 = $p < 0.001$ (two tailed matched t-test).

Figure 3 displays a combined anthropometric and craniofacial profile of 19 variables indicating the percent difference from the overall mean comparing 13 white fra X males with high fra X chromosome expression (>30%) with 13 age-matched white fra X males with low fra X expression (<30%). A relatively flat profile was identified with an overlap of most variables and no significant differences for any of 19 variables. Table 3 shows the average ± standard deviation and t-test value for each variable for the two groups of age-matched fra X males with either high or low fra X chromosome expression.

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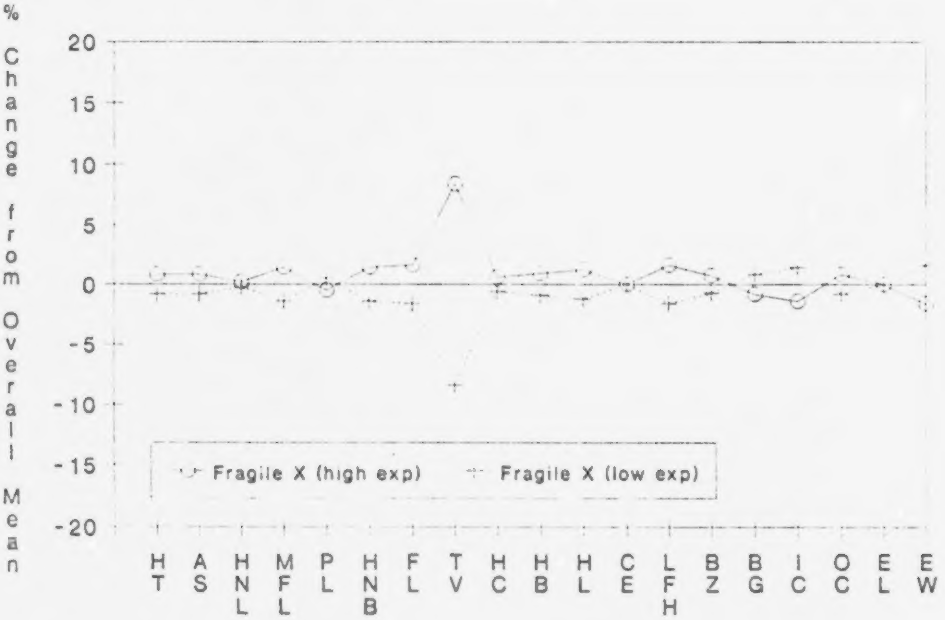

Figure 3. Anthropometric/craniofacial profile indicating percent difference from overall mean for 13 white fragile X syndrome males with high fra X chromosome expression (>30%) matched for age with 13 white fragile X syndrome males with low fra X expression (<30%). HT = height, AS = arm span, HNL = hand length, MFL = middle finger length, PL = palm length, HNB = hand breadth, FL = foot length, TV = testicular volume, HC = head circumference, HB = hand breadth, HL = head length, CE = cephalic index, LFH = lower face height, BZ = bizygomatic diameter, BG = bigonial diameter, IC = inner canthal distance, OC = outer canthal distance, EL = ear length, EW = ear width.

DISCUSSION

An anthropometric pattern profile did emerge between mentally retarded males with or without fra X syndrome with the fra X males having greater dimensions for all anthropometric variables and significant differences for height, sitting height, arm span, hand length, middle finger length, hand breadth, foot length and foot breadth and testicular volume with the greatest differences for foot length and foot breadth. Significant differences were identified with greater head circumference, head breadth, lower face height, ear length and ear width in the fra X males, supporting an elongated face, large ears and macroorchidism in fra X males while bizygomatic

Table 3
Anthropometric and Craniofacial Patterning Data from 13 White Fragile X Males with High Fra X Expression (>30%) matched for age with 13 White Fragile X Males with Low Expression (<30%)

Variables	Fragile X Males With High Expression (Mean ± SD)	Fragile X Males With Low Expression (Mean ± SD)	Matched T-Test Values
Height (cm)	157.7 ± 30.4	155.3 ± 28.9	0.84
Arm span (cm)	161.6 ± 26.1	159.2 ± 21.2	0.61
Head circumference (cm)	55.8 ± 3.1	55.2 ± 3.5	0.71
Head breadth (cm)	14.8 ± 0.7	14.6 ± 1.0	1.04
Head length (cm)	19.4 ± 1.2	19.0 ± 1.3	0.95
Lower face height (cm)	11.8 ± 1.1	11.4 ± 1.7	0.95
Bizygomatic diameter (cm)	12.5 ± 1.2	12.3 ± 1.1	0.49
Bigonial diameter (cm)	10.1 ± 1.1	10.2 ± 1.0	0.50
Inner canthal distance (cm)	2.8 ± 0.4	2.9 ± 0.3	0.37
Outer canthal distance (cm)	8.7 ± 0.8	8.5 ± 0.9	0.51
Ear length (cm)	6.8 ± 0.9	6.9 ± 0.8	0.76
Ear width (cm)	4.1 ± 0.6	4.2 ± 0.5	0.98
Hand breadth (cm)	7.4 ± 1.3	7.2 ± 1.6	0.98
Middle finger length (cm)	7.4 ± 1.2	7.2 ± 1.3	1.50
Hand length (cm)	16.9 ± 2.8	16.9 ± 2.9	0.15
Palm length (cm)	9.5 ± 1.8	9.6 ± 1.7	0.60
Foot length (cm)	24.8 ± 3.2	24.0 ± 2.0	1.32
Testicular volume (ml)	46.4 ± 30.7	39.2 ± 32.0	0.71
Cephalic index	76.0 ± 26.0	77.0 ± 27.0	0.38

T-test values are not significant ($p>0.05$; two-tailed)

diameter and inner canthal distance were significantly larger in the mentally retarded males without the fra X. Better characterization of other features occurred in this study and under recognized physical parameters were identified in fra X males when compared with mentally retarded males without the fragile X syndrome. These included large head dimensions with an increased head circumference and head breadth, but interestingly, a smaller minimal frontal diameter with relative hypotelorism (indicated by narrowing of the inner canthal distance and larger outer canthal distance), broad palpebral fissures, an increased ear width relative to ear length and larger feet in the fra X males. A narrow face relative to face length in fra X patients is also supported by our data. Therefore, several head and face parameters (e.g., head breadth, inner and outer canthal distances, ear width) may be useful as previously under recognized characteristics for screening individuals for the fra X.

In order to identify if physical parameters differ in fra X males with high fra X chromosome expression (>30%) compared with low expression (<30%), a combined anthropometric and craniofacial patterning analyses were performed on 19 variables in 26 fra X males matched for age and race. In our study, a flat profile (excluding testicular volume which was not statistically significantly different) was observed for both the high or low expressing fra X males and no significant differences were found. Recent statistical analysis with cytogenetic and DNA data indicates a significant positive correlation between high fragile X chromosome expression and large DNA alterations, i.e., increased number of DNA inserts of CGG trinucleotide repeats (de Vries et al., 1992). Therefore, our fra X males with high expression probably have larger DNA alterations than those patients with low expression. There appears to be no difference in physical parameters in our study in fra X males with or without high fra X chromosome expression.

The results of our research are encouraging in selecting variables useful for screening males for the fra X and in better characterizing the features of this fascinating syndrome. Additional research is needed with a larger sample of males with or without the fra X not only for confirmation of our results, but also to correlate these physical parameters with alterations of the FMR-1 gene. Our research illustrates the utility of anthropometry in the evaluation of mentally retarded patients and offers a comprehensive approach for screening males for the fra X and selecting those individuals for cytogenetic and/or molecular genetic testing.

ACKNOWLEDGEMENTS

We thank Pamela Grimm for expert preparations of the manuscript; the Tennessee Department of Mental Health and Mental Retardation grant and Clinical Research Center grant, Meharry Medical College, Nashville, Tennessee (M.G.B., R.P., D.N.S.) and NICHD grant HD11624 (M.S.W., W.R.B.) for financial support; Dr. Ellen Roback, Karen King, Andy Allen, June Burns, Roberta Thomas and other personnel at Cloverbottom Developmental Center, Nashville for their assistance in the collection of data and Lora Miller, Judy Haynes and Weitong Zhu for technical assistance.

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ABNORMAL CYCLIC AMP PRODUCTION IN TISSUES FROM INDIVIDUALS WITH FRAGILE X SYNDROME

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ABSTRACT

Learning and short-term neuronal memory appear to depend, in part, upon activation of second messenger systems such as the cAMP cascade. Abnormal function of adenylate cyclase subunits results in diminished cAMP production and learning deficiency in Drosophila mutants and in humans with pseudohypoparathyroidism type Ia. We have studied cAMP production in several tissues from fragile X patients and controls utilizing 1-isobutyl-3-methylxanthine (IBMX) to inhibit phosphodiesterase, prostaglandin E_1 (PGE_1) to stimulate cAMP production via a receptor-dependent mechanism, and forskolin (FSK) to directly activate the catalytic subunit of adenylate cyclase. In platelets, cAMP production in IBMX, PGE_1+IBMX, and FSK+IBMX was 50% ($p<0.05$), 65% ($p=0.001$), and 53% ($p=0.001$), respectively, in the fragile X group (N=31) relative to the control group (N=57). Basal cAMP levels in the absence of drugs were not significantly different between fragile X and control groups. Cyclic AMP production was not statistically different from controls in patients with non-specific mental retardation (N=16) or autistic disorder (N=4). There was no effect of age or sex on cAMP production.

Dose response curves suggested diminished maximal response rather than alterations in potency of the reagents. FSK-stimulated cAMP production in platelets correlated significantly with Xq27.3 fragile site percentages in lymphocytes from the same patients. In lymphoblastoid cells (LCLs) created from fragile X patients (N=13), cAMP production in PGE+IBMX, and FSK+IBMX was 65% and 55%, respectively, of cAMP production in control LCLs (N=4). In fragile X fibroblast lines (N=11), cAMP production in PGE1+IBMX was 68% of that in control lines (N=4) although data was quite variable and this difference was not statistically significant.

These data suggest that a defect in cAMP production exists in several tissues from fragile X individuals and may play a role in mediating cognitive and neurobehavioral dysfunction in these patients. Variability in cAMP data are consistent with wide clinical variability among fragile X patients and may reflect variation in expression of FMR-1 in different fragile X individuals and tissues.

INTRODUCTION

The cyclic AMP (cAMP) cascade is a second messenger cascade which is thought to regulate neuronal functions and produce short-term sensitization (or neuronal memory) through the following mechanism. Receptors bind neurotransmitters or other neuroactive ligands causing interaction of receptor proteins with signal-transducing GTP-binding regulatory subunits (G_s or G_i for stimulatory or inhibitory, respectively) of adenylate cyclase. Regulatory subunits then interact with the catalytic subunit of adenylate cyclase to increase or decrease production of cAMP. Intracellular cAMP binds to regulatory subunits of cAMP-dependent protein kinases (PK-A) and activates these kinases resulting in phosphorylation and activation (or deactivation) of their protein or enzyme substrates and thus, modification of neuronal synaptic properties. Phosphodiesterases inactivate cAMP by hydrolysis and phosphatases dephosphorylate protein substrates.

The cAMP cascade can produce long-term changes in neuronal properties and responsiveness via induction of phosphorylation of transcription factors which bind AP-2 or CRE consensus sites thereby enhancing or inhibiting transcription of selective groups of genes (Walton & Rehfuss, 1992). This is a process thought to play a role in long-term sensitization in simple organisms and in learning in higher organisms. Therefore, biochemical lesions affecting regulation or activity of this cascade would be expected to produce learning or memory deficits. In fact, disruption of cAMP cascade function in lower organisms and in humans does appear to produce "learning" problems.

In *Aplysia* blockade of cAMP synthesis or injection of inhibitors of PK-A prevents short-term sensitization or desensitization of the gill withdrawal reflex (which is thought to occur via PK-A-dependent phosphorylation of a potassium channel) as well as longer-term changes in protein synthesis which are thought to lead to long-term facilitation (Kendel & Schwartz, 1982; Barzilai et al., 1989).

In *Drosophila* selection for mutants which are learning deficient but have normal motor and sensory function has identified several mutants with decreased activity of cAMP cascade protein (Dudai, 1986)) The Dunce mutant is deficient in phosphodiesterase; Rutabaga has diminished activity of the catalytic subunit of adenylate cyclase; Ddc is dopa-decarboxylase deficient and produces lowered amounts of dopamine for neurotransmission; Turnip has diminished activity of the G_s regulatory subunit of adenylate cyclase.

In humans, patients with type Ia pseudohypoparathyroidism have been shown to have diminished activity of the G_s regulatory subunit of adenylate cyclase (Farfel et al., 1981) due to a variety of mutations within the $G_s\alpha$ gene (Levine et al., 1988; Patten et al., 1990). These patients, unlike patients with other forms of pseudohypoparathyroidism, display a variable degree of learning disorder or mental retardation (Farfel & Friedman, 1986), presumably due to deficiency of $G_s\alpha$-mediated signal transduction in brain. Patients with myotonic dystrophy have recently been shown to have a mutation affecting a gene for a cAMP-dependent protein kinase (Brook et al., 1992). These patients may have substantial and sometimes progressive cognitive difficulties, possibly on the basis of interruption of normal cAMP-mediated signal transduction.

Other second messenger cascades may be implicated in human developmental disorders. Patients with autistic disorder (particularly the hyperserotoninemic subgroup) demonstrate diminished fenfluramine-stimulated prolactin release from CNS neurons, 5HT-mediated platelet aggregation, and B_{max} for agonist but not antagonist binding to $5HT_2$ sites on platelet membranes

(McBride et al., 1989).

I have studied cAMP metabolism in several tissues from patients with fragile X syndrome, non-specific mental retardation, autistic disorder, and normal controls in order to determine whether biochemical disruption of this cascade might play a role in the cognitive dysfunction seen in these patients. Platelets were utilized initially because they provide the best model for CNS cAMP systems in that they, like brain, possess high levels of adenylate cyclase activity, are a non-dividing tissue, and utilize several second messenger systems which interact to regulate function. Other tissues were then studied to confirm the observation of diminished cAMP production in platelets.

METHODS AND PATIENTS

Patients with fragile X syndrome were recruited from the Pediatric Neurology clinic at the University of Chicago or from the Fragile X Association of America. All patients in the fragile X group (age range 4-50) had a clinical phenotype consistent with the syndrome and confirmation of the diagnosis by cytogenetic testing demonstrated a significant percentage (>10%) of folate-sensitive fragile sites at Xq27.3. Many patients have now had additional confirmation of diagnosis via DNA testing on Southern blots. Patients in the mentally retarded group (age range 3-45) were recruited from the Pediatric Neurology clinic and had cognitive impairment similar to fragile X patients but a clinical picture which was not classical for the fragile X syndrome, negative cytogenetic testing, and no other specific diagnosis of etiology for mental retardation. Patients with autistic disorder were recruited from the Child and Adolescent Psychiatry Department. The diagnosis of autistic disorder was based on the Autism Diagnostic Interview. Controls were unmedicated and normally intelligent with an age range of 6-60.

Blood was obtained from patients and controls in the same assay batches. Platelets were isolated from blood samples as described previously (Berry-Kravis & Huttenlocher, 1992) and in some cases lymphocytes were isolated on Ficoll-Paque gradients, transformed with Epstein Barr virus and grown in culture. Fibroblast cultures were grown from skin biopsies. Cyclic AMP production experiments were performed as described (Berry-Kravis & Huttenlocher, 1992). Briefly, platelet/cell suspensions or fibroblasts were incubated with appropriate drugs for 30 minutes. 1-Isobutyl-3-methylxanthine (IBMX) was used to inhibit phosphodiesterase, prostaglandin E_1 (PGE_1) to measure receptor-dependent stimulation of cAMP production, and forskolin (FSK) to directly activate the catalytic subunit of adenylate cyclase. Reactions were stopped by TCA precipitation, protein removed by centrifugation, and TCA removed from cAMP-containing supernatants by ether extraction. Protein was measured by the method of Lowry et al. (1951) and cAMP by RIA (Brooker et al., 1979).

All assays were performed in triplicate. When a patient or control was assayed more than once, values were averaged. Cyclic AMP measurements in all figures are plotted as picomoles per milligram of protein. Error bars on all figures represent S.E.M.s. Values of p were determined by the student's t-test.

RESULTS

Cyclic AMP production in platelets was studied in fragile X patients (N=31; from 23 different families), mentally retarded patients (N=16), autistic patients (N=4), and normal controls (N=57) (Figure 1). PGE_1 (10μM)- and FSK (50μM)-stimulated cAMP production in the presence of IBMX (1mM) are significantly lower in the fragile X group (65 and 53% respectively, $p=0.001$); and cAMP production in IBMX alone is 50% of control ($p<0.05$). Basal levels are 72% of control (NS). Cyclic AMP production is not significantly different from controls in the mentally retarded (in IBMX, PGE_1 + IBMX, and FSK + IBMX it is 117, 86, and 103%, respectively) and autistic (in IBMX, PGE_1 + IBMX, and FSK + IBMX it is 146, 102, and 84%, respectively) groups. Inhibition of cAMP production with epinephrine is similar to controls in the fragile X group (data not shown). When fragile X patients who were treated with medication (N=9) are eliminated from the analysis, cAMP production in the fragile X group is 53% of control in both PGE_1 and FSK and statistical significance is unchanged.

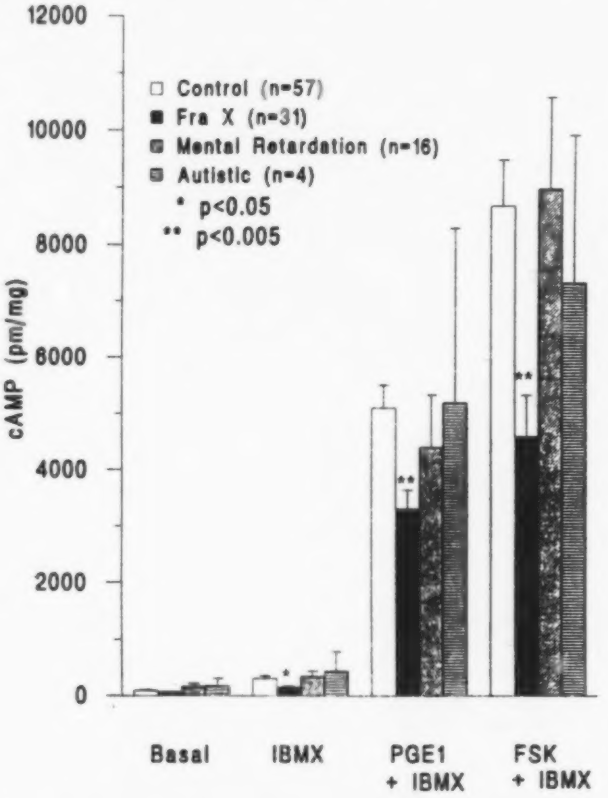

Figure 1. Cyclic AMP production in platelets from controls and patients with fragile X syndrome, mental retardation, or autistic disorder. IMX was 1 mM, PGE_1 was 10 μM and FSK was 50 μM. *$p<0.05$, **$p=0.001$.

No measure of cAMP production is statistically different between control males (N=32) and females (N=25), although values tend to be slightly higher in the male group (Berry-Kravis and Sklena, 1992). If comparisons are made only to the all-male control group (all fragile X patients are male) there would be a slight increase in the magnitude (for all measures of cAMP production) of the cAMP production defect seen in the fragile X group with no change in statistical significance. Likewise, no differences in cAMP production values between males and females in the mentally retarded group were seen (Berry-Kravis and Sklena, 1992). No effect of age is seen on any measure of cAMP production in the control, fragile X, or mentally retarded groups (Berry-Kravis and Sklena, 1992).

Averaged dose response curves in IBMX with either PGE_1 or FSK show diminished maximal response but similar curve shapes for the fragile X group as compared to the control group (Figure 2), suggesting that lowered production in the fragile X samples is not due to diminished drug potency. Statistical significance is seen for virtually all drug concentrations with fragile X samples always having lower cAMP production. Averaged dose response curves for the control and mentally retarded groups are not significantly different (Figure 3).

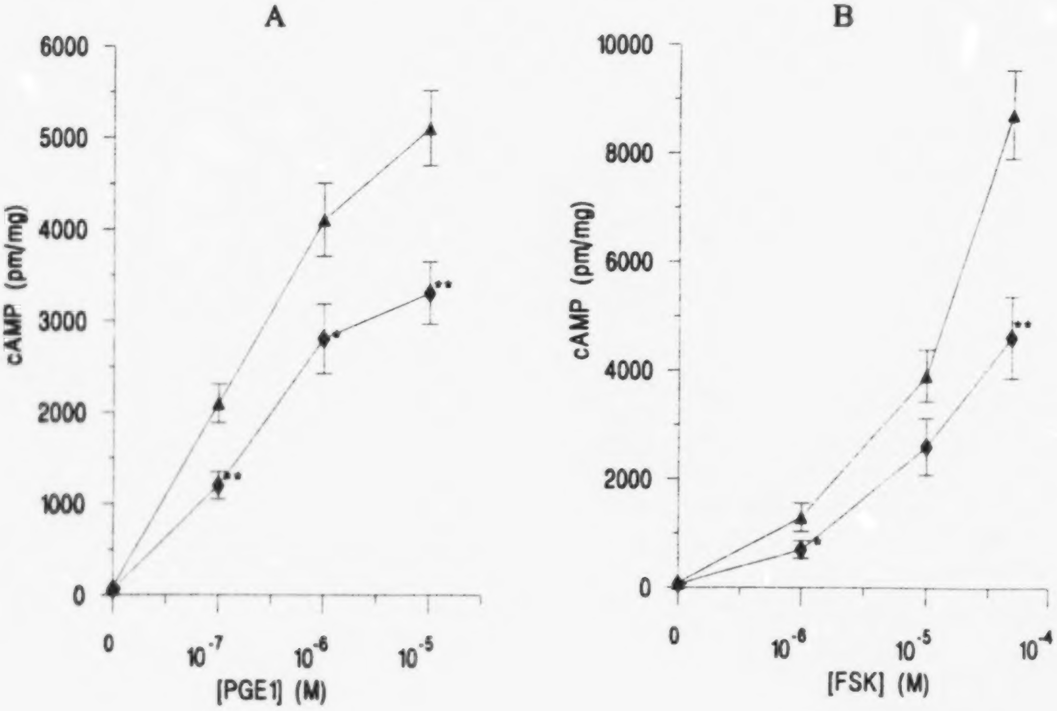

Figure 2. Averaged cAMP production in platelets from control (▲) and fragile X (◆) groups in the presence of IBMX (1mM) and increasing doses of PGE_1 (A) and FSK (B). *$p<0.05$, **$p<0.005$

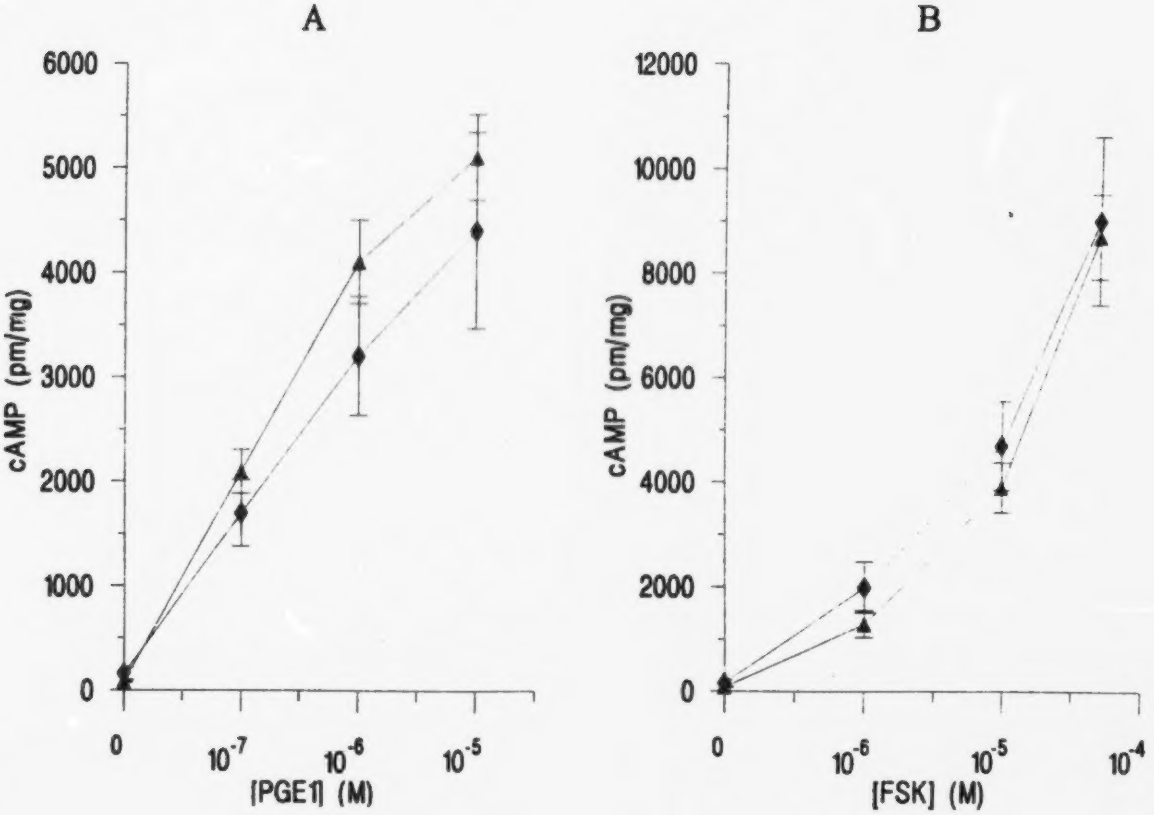

Figure 3. Averaged cAMP production in platelets from control (▲) and mentally retarded (◆) groups in the presence of IBMX (1mM) and increasing doses of PGE_1 (A) and FSK (B).

In order to eliminate variables related to diet/drugs etc. and to demonstrate that cAMP production findings could be demonstrated in more than one tissue, cAMP metabolism was examined in control and fragile X lymphoblastoid cells (LCLs) as well as a limited number of control and fragile X fibroblast cultures. Figure 4 shows data from LCLs for a single assay. Cyclic AMP production is diminished in fragile X cells (N=7) relative to controls (N=4) in IBMX alone (61%, $p<0.05$), PGE_1+IBMX (61%, $p<0.01$), and FSK+IBMX (66%, $p<0.05$). The table shows averaged data for fragile X (N=13) and control (N=11) LCLs from multiple (all) assays. High interassay variability lowers statistical significance but PGE_1- and FSK-stimulated cAMP production in fragile X lines are 65% and 55% of control with $p<0.05$ and $p=0.1$, respectively.

In a separate assay, LCLs from cytogenetically positive fragile X female carriers with full mutations (N=3) showed lower cAMP production than those from cytogenetically negative females with premutations (N=3). No correlation was seen between LCL growth rate or passage number and cAMP production in any assay.

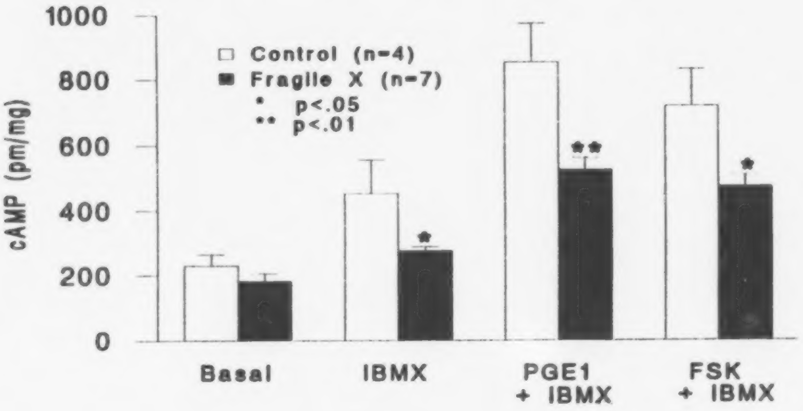

Figure 4. Cyclic AMP production in LCLs from controls and patients with fragile X syndrome. IBMX was 1 mM, PGE_1 was 10 μM, and FSK was 50 μM. *p<0.05, p<0.01.

Table 1
Cyclic AMP Production in LCLs
From Control and Fragile X Individuals

Reagents	cAMP (pmol/mg) Controls (n=11)	 Fra X (n=13)	Fra X/C (%)	p
Basal level	100 ± 31	104 ± 25	104	0.9
IBMX(1mM) alone	204 ± 64	178 ± 26	87	0.7
PGE1(10uM)+IBMX	601 ± 97	390 ± 45	65	0.05
FSK(50uM)+IBMX	604 ± 177	335 ± 41	55	0.1

No statistically significant differences are seen between cAMP production in control (N=4) and fragile X (N=4) fibroblasts (Figure 5). Data from this cell type was extremely variable, and although there is a trend toward lower cAMP production (61%) in PGE_1+IBMX in fragile X cells, larger groups are needed to assess this effect.

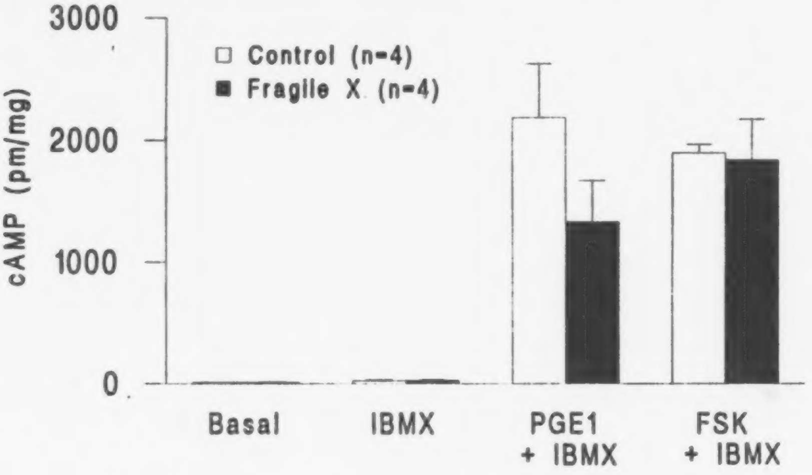

Figure 5. Cyclic AMP production in fibroblasts from control and fragile X individuals. IBMX was 1 mM, PGE_1 was 10 μM, and FSK was 50 μM.

When Xq27.3 fragile site percentages from lymphocytes in the 14 study patients who had cytogenetic testing done at the University of Chicago cytogenetics laboratory (to maintain constancy of folate deprivation conditions) are plotted against FSK-stimulated cAMP production for these patients (Figure 6), a significant negative correlation is seen with r=0.65 and p=0.01.

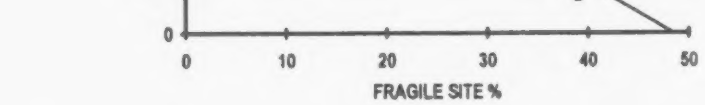

Figure 6. Correlation of Xq27.3 fragile site percentages and 50 μM FSK-stimulated cAMP production in 1 mM IBMX.

DISCUSSION AND CONCLUSIONS

Based on this work, I conclude cyclic AMP production is decreased in both platelets and lymphoblastoid cells from fragile X patients relative to controls. This effect is not seen in other mentally retarded patients. The defect is not as evident in fibroblasts although more cell lines are needed for study and there is a trend toward lower PGE_1-stimulated cyclic AMP production in fragile X fibroblasts. The pattern of decreased cAMP production with FSK-stimulated cAMP production as much or more diminished than PGE_1-stimulated cAMP production suggests decreased activity of a catalytic subunit of adenylate cyclase rather than defective receptor coupling. Decreased activity of the catalytic unit could be due to a lower amount of the catalytic unit protein, a change in the membrane environment or a change in proteins which associate with the catalytic unit.

Fragile X patients demonstrate a variety of neuropsychological problems including attentional difficulties, mood disorder, and psychosis which have suggested frontal lobe dysfunction. Cognitive profiles, particularly in fragile X females, have pointed to parietal lobe dysfunction as well (Grigsby et al., 1990). Coordination problems have suggested cerebellar dysfunction and the cerebellar vermis has been reported to be smaller in fragile X individuals than in controls (Reiss et al., 1991). The multifocal nature of CNS dysfunction in fragile X syndrome has been difficult to attribute to a single neuronal or neurotransmitter system. The cAMP cascade, like other second messenger systems, is involved in regulation of multiple neurotransmitter systems in different brain areas, and most likely regulates the ability of different neurotransmitter systems to regulate each other. Dysfunction of the cAMP cascade would thus, be expected to produce multifocal cognitive problems such as seen in fragile X syndrome.

One specific problem which is quite pronounced in fragile X patients is hypersensitivity to sensory stimuli and to the environment in general. This appears to result from difficulty with habituation to typical environmental stimuli. Interestingly, the processes of desensitization to non-noxious stimuli and habituation of reflex responses in serotoninergic sensory neurons of *Aplysia* appear to be at least partially dependant on stimulation of cAMP production. The cAMP cascade has been implicated in short-term sensitization and memory functions in *Aplysia* (Kandel & Schwartz, 1982) and *Drosophila* (Dudai, 1986), while fragile X patients demonstrate particular weakness in short-term memory subtests of neuropsychological testing profiles (Freund & Reiss, 1991). Thus, impaired regulation of the cAMP cascade might be expected to produce problems commonly seen in fragile X patients, such as sensory hypersensitivity, difficulty with habituation, and short-term memory deficits.

Other observations which support a link between cognitive and cAMP cascade dysfunction in fragile X syndrome include the report of abnormal appearance of Golgi impregnated dendritic spines and decreased synaptic density in fragile X brain (Wisniewski et al., 1991). Second messenger systems are thought to participate in synaptogenesis through regulation of transcription of factors important for outgrowth of processes and formation of interneuronal connections.

A mutation in a gene encoding a cAMP-dependent protein kinase has recently been found to occur in patients with myotonic dystrophy, suggesting that cAMP cascade dysfunction may be important in producing cognitive impairment in this condition (Brook et al., 1992). Further, findings on neuroimaging studies (mild increase in ventricular size) and cognitive profiles in patients affected with myotonic dystrophy resemble those from fragile X patients. These similarities might be expected to derive from separate biochemical lesions within the same pathway or cascade.

Although the cAMP cascade acts in cellular regulation in multiple organs and major abnormalities of function might be expected to produce multi-organ problems, the CNS is likely to be more highly sensitive to subtle cAMP cascade dysfunction. Cyclic AMP cascade proteins and enzyme activities are expressed in higher amounts in brain than in most tissues. Also, CNS-specific phosphodiesterases (Beavo, 1989), cyclases (Bakalyar & Reed, 1990; Krupinski et al., 1989), and protein kinases (Nestler & Greengard, 1984) have been described. The cAMP cascade and other second messenger systems no doubt require very fine "tuning" within the CNS to participate in the process of normal human learning and memory.

The gene which is thought to mediate mental retardation in fragile X syndrome, FMR-1, does not code for a cAMP cascade protein but may code for a nuclear protein (Verkerk et al., 1991). If so, the FMR-1 gene may regulate cAMP cascade function transcriptionally either primarily by altering transcription of cAMP cascade protein(s) or by altering transcription of a protein with a regulatory (secondary) effect. If not a nuclear protein, there is some evidence to suggest that the FMR-1 protein may be an extracellular signal peptide (Yu et al., 1992) and in this case might directly or indirectly alter cAMP cascade metabolism via a cellular mechanism.

Although in most fragile X patients the FMR-1 mRNA is not expressed at all, there is a subgroup of 10-20% of patients who appear to express a varying amount of FMR-1 mRNA (Pieretti et al., 1991). These patients appear as "methylation mosaics" in genomic DNA (Pieretti et al., 1991; Oberle et al., 1991). The negative correlation of fragile sites with cyclic AMP production in fragile X platelets may indicate that cAMP production is less decreased in "methylation mosaics" (who make some FMR-1 mRNA) if one assumes that "methylation mosaic" patients will tend to be lower fragile site expressors.

Comparison of cAMP production data and mRNA levels for cAMP cascade proteins in methylation mosaic (+ for FMR-1 mRNA) and non-mosaic cell lines (- for FMR-1 mRNA) will be important to establish a more definitive relationship between FMR-1 and the cAMP cascade. So far, 5 of the fragile X LCLs from the experiments above have been assayed for FMR-1 mRNA via multiplex RT-PCR (Pieretti et al., 1991) and are negative for FMR-1 mRNA. Assay of a much larger series of lines should generate FMR-1 mRNA(+) and FMR-1 mRNA(-) subgroups. Alternative means of demonstrating a link between FMR-1 expression and cAMP cascade function include demethylation of FMR-1 in fragile X LCLs with 5-azacytidine and comparison of cAMP production in demethylated and non-demethylated subclones from the same patient. Eventually, expression of normal FMR-1 mRNA in cell lines which are FMR-1 mRNA (-) via transfection with plasmid containing FMR-1 cDNA may allow direct evaluation of cAMP/FMR-1 relationships. Finally, it will be important to confirm the cAMP production defect in fragile X brain tissue or embryonic fragile X neuroglial cells in culture.

If a link can be clearly established between FMR-1 and the cAMP production defect seen in fragile X tissues in this study, it will be likely that altered cAMP cascade regulation in fragile X brain contributes to cognitive and neurobehavioral dysfunction in fragile X patients.

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THE MEANING OF SEIZURES, PAROXYSMAL ACTIVITY AND HYPERACTIVITY IN SUBJECTS WITH FRAGILE X SYNDROME

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ABSTRACT

The aim of this study was to investigate the relationship between EEG abnormalities and clinical-laboratory findings in the fragile X syndrome. We examined 52 fragile X male subjects (aged 1-57 years) and we considered the percentage of fragile X chromosomes in their karyotype, the presence of seizures, the presence of paroxysmal activity in their EEG and the presence of hyperactivity. A significant positive correlation was found between paroxysmal activity and hyperactivity in the younger subgroup (aged 1-13 years), and between seizures and percentage of lymphocytes with the fragile X chromosome (in the whole group). The correlation between EEG paroxysmal activity and hyperactivity supports the hypothesis of the presence of cortical hyperexcitability in subjects with the fragile X syndrome. The more frequent presence of seizures in subjects with higher fragile X percentage suggests that a more pronounced genetic involvement is present in these individuals.

Key Words: seizures, epilepsy, EEG, paroxysmal activity, hyperactivity, fragile X syndrome.

INTRODUCTION

Since the recognition of the fragile X syndrome, additional signs and symptoms have been described (Turner et al., 1986; Neri et al., 1988; Brown et al., 1991). Thus, to the classical signs of macroorchidism, mental retardation and connective tissue dysfunction have been added central nervous system (CNS) dysfunction, such as epilepsy (Finelli et al., 1985; Wisniewski et al., 1991), hyperactivity (Fryns et al., 1984), tantrums, violent outbursts, aggression, poor eye contact (Hagerman, 1987; Hagerman & Silverman, 1991), and a specific electroencephalographic (EEG) pattern (Musumeci et al., 1988).

The study of the symptomatology of the fragile X syndrome suggests certain conclusions: a) symptoms are emphasized by authors depending on particular interests and competence, and b) not all the fragile X subjects show the same symptoms (Hagerman, 1987). Significant physical signs like macroorchidism and behavioral signs, such as outbursts and hyperactivity, may be absent (Butler et al., 1991). This suggests that the fragile X phenotype is variable, probably in relation to a variable genetic involvement. For example, different signs and symptoms could be expressions of a specific neurotransmitter alteration.

Few studies demonstrate correlations between different symptoms of the fragile X syndrome (Cronister et al., 1991). This paper evaluates the signs and symptoms detected in a group of fragile X subjects and correlates them with the cytogenetic status.

MATERIALS AND METHODS

For this study, 52 random male subjects, 15 of whom were epileptic, with the fragile X syndrome (diagnosis confirmed by karyotyping) were evaluated with an EEG assessment. The mean age of the group was 15.2 years (range 1-57 years). Additional data collected in these patients included age, cytogenetic results, presence and type of seizures, presence of hyperactivity judged clinically according to DSM III-R criteria for attention deficit hyperactivity disorder and the Conners Rating Scale and anticonvulsant medication used.

The Chi-square test was used to analyze correlations between paroxysmal EEG activity, history of seizures and the other parameters. With the term paroxysmal activity, in this study, we indicated the particular EEG pattern, usually observed during sleep that has been previously described (Musumeci et al., 1988).

Moreover, for some parameters which change from childhood to adulthood, such as hyperactivity, the patients were subdivided into two subgroups according to their age; one subgroup comprised subjects aged 1-13 years, the second was formed by subjects older than 13 years. For the reading of the EEGs the neurologists were blind to the clinical information, including the diagnosis of each patient.

RESULTS

Fifteen (28.8%) subjects out of 52 were found with a history of epileptic seizures, also 15 (28.8%) showed paroxysmal activity in their EEG, 13 of them belonged to the younger subgroup. It is interesting to note that only five subjects had both seizures and paroxysmal activity.

There were significant correlations between history of seizures and the frequency of fragile X cells in the karyotype (Table 1), and between paroxysmal EEG activity and hyperactivity in the younger subgroup (Table 2).

Moreover, only five subjects out of 19 (younger subgroup) had moderate to severe hyperactivity and were taking antiepileptic drugs (carbamazepine). Two of the subjects had paroxysmal activity in their EEG.

Table 1
Correlation Between History of Seizures and Percentage of Fragile X Cells in the Karyotype

	History of Seizures	
Frequency of Fragile X Cells	yes	no
<10%	3	16
10-30%	7	20
>30%	5	1

Chi-square = 10.369 p <0.006

Table 2
Correlation Between Hyperactivity and Paroxysmal EEG Activity in Subjects Aged 1-13 years

	Hyperactivity	
Paroxysmal EEG Activity	absent or mild	moderate to severe
absent	11	8
present	2	11

Chi-square = 5.783 p <0.02

DISCUSSION

As stated above, it is known that the phenotype of the fragile X syndrome can be variable and this appears to be related to variable genetic involvement. The hypothesis of Laird (1987) and the new DNA findings support this point of view (Oberle et al., 1991). In transmitting males with less than 200 CGG repeats, there is little or no clinical expression of disease and usually no fragile X expression cytogenetically. In affected males and in females with high expression of fragile X, the insertion is much larger (600 to 1000 CGG repeats).

The finding of a significant correlation of seizures with a higher percentage of fragile X cells suggests that epilepsy occurs when the genetic involvement is more severe. Thus, the percentage of fragile X cells in the karyotype has, to some extent, a predictive value with respect to the occurrence of seizures. Moreover, epilepsy in the fragile X syndrome occurs with greater involvement of the CNS. The presence of epilepsy in 28.8% of our subjects suggests that this is a fundamental sign of the syndrome. Mental retardation is more common in affected males and in heterozygous females with more than 2% of fragile X expression (Cronister et al., 1991).

The fragile X syndrome represents a genetic model of epilepsy. It also provides information concerning the inheritance of the EEG trait similar to that of the benign epilepsy of childhood with centrotemporal spikes (Musumeci et al., 1988) which in 41 percent of the young patients presented the characteristic EEG pattern and five out of 15 with epilepsy showed the pattern in only one recording. In fragile X patients, the EEG pattern is not detectable in all recordings, similar to benign epilepsy of childhood with centrotemporal spikes (Bray & Wiser, 1965). Activation methods or repeated recordings are necessary in order to detect the presence of paroxysmal activity.

Hyperactivity was a notable problem in early reports of fragile X males (Mattei et al., 1981) and further documented by Finelli et al. (1985) and Hagerman (1987). Fryns et al. (1984) emphasized the disappearance of hyperactivity after puberty. Similarly, follow-up studies on the fragile X EEG pattern (Musumeci et al., 1991) demonstrated its disappearance after puberty. There are several reports in the literature on the association between presence of spikes or paroxysms in the EEG and hyperactivity. The latter, together with behavior disturbances, is frequently detected also in patients with paroxysmal EEG activity without seizures (Fois & Lippi, 1970; Cavazzutti et al., 1980), and in fragile X subjects with normal IQ scores (Hagerman et al., 1985).

At the moment, it is not clear whether long-term therapy with phenobarbital is responsible for the occurrence of hyperactivity in epileptic children or not. In fact, Wolfe and Forsythe (1978) found that 42% of 109 children treated with phenobarbital developed hyperactivity which disappeared in 73% and improved in all of them after discontinuation. Because of this data, we would like to emphasize that phenobarbital should not be utilized as a first-choice drug for antiepileptic therapy in fragile X subjects.

Our results, in need of further confirmation, show a significant correlation between hyperactivity and presence of paroxysmal activity in the EEG. Perhaps these two features are related to similar alterations in CNS structure or neurotransmitter dysfunction caused by the complete or partial absence of the FMR-1 protein. Further studies in the field of molecular genetics, brain structure and neurochemistry in fragile X will hopefully answer these questions.

ACKNOWLEDGEMENTS

This research was supported by The Children's Kempe Research Center at The Children's Hospital, Denver, Colorado and by the Oasi Institute, Troina, Italy.

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OCULO-VISUAL DYSFUNCTION IN FRAGILE X SYNDROME

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ABSTRACT

Fragile X syndrome (fra X) has numerous associated cognitive, physical, and behavioral abnormalities. The studies that have been published and the information given during this symposium indicate that several eye problems occur in fragile X. These associated problems include moderate to high refractive error and strabismus. All children with fra X should receive routine vision care by an appropriate eye care professional. It is also recommended that any child with mental retardation of unknown etiology who presents with strabismus be screened for fragile X syndrome.

INTRODUCTION

Various checklists have been developed to assist in the diagnosis of fra X in those who present with mental retardation without a known etiology (Butler et al., 1991; Hagerman, 1991). None of these observational techniques note the importance of oculo-visual abnormalities in this special population. The diagnosis and treatment of visual dysfunction in children with fra X has been ignored by most researchers in both the ophthalmic and non-ophthalmic scientific community with few reporting their findings in the literature. Although this may be due to the absence of ocular disorders in this population, the studies that have been published and the information given during this symposium indicate that several eye disorders must be considered associated conditions of fragile X syndrome as well (Table 1) (Maino et al., 1991; Maino et al., 1990; Storm et al., 1987; King et al., 1992).

Maino et al. (1991), Storm et al. (1987), and Flood & Sanner (1985) have noted a high incidence of strabismus, refractive errors, and other ocular abnormalities. Maino evaluated 30 subjects and found approximately 30% exhibited strabismus. Of those with a heterotropia, 70% demonstrated esotropia. Nystagmus was present in 13% of the population with 59% having hyperopia, 17% myopia, and 22% astigmatism of at least one diopter. No major ocular health problems were identified in any of the subjects.

Table 1
Oculo-Visual Abnormalities of Fragile X Syndrome

Refractive Error***	**Oculo-motor**	**Other**
Hyperopia	Strabismus***	Amblyopia
Myopia	Esotropia	Ptosis
Astigmatism	Exotropia	Epicanthal folds
	Vertical deviations	
	Nystagmus	

***Indicates associated ocular anomalies

King et al. (1992) noted that strabismus was also the most common finding in his patients with more than 40% demonstrating an eye turn (50% esotropia and 50% exotropia). Ten percent of the population exhibited amblyopia. Significant refractive errors were seen in 25% of the fragile X patients. Nystagmus, ptosis, convergence insufficiency, and accommodative insufficiency were also noted.

The high incidence of strabismus in this population puts the fra X child at risk for the development of amblyopia and the possibility of permanent vision loss. Learning related vision disorders, such as uncorrected hyperopia, convergence insufficiency and accommodative insufficiency (Solan, 1991) may inhibit progress in the child's educational and rehabilitation programs.

THE VISION EXAMINATION

The examination techniques used for this population must vary according to the cognitive and behavioral function of the patient. The use of hand held instruments (i.e., tonometers, biomicroscopes) will allow the practitioner the flexibility needed to gather as much clinical information as possible (Table 2). The assessment of visual acuity requires the utilization of behavior observation techniques (Teller Acuity Cards) or optotypes specifically designed for young or non-communicative patients (Broken Wheel Acuity Test, Lighthouse Flash Card Test, Allen Cards) (Schlange & Maino, 1993).

The assessment of the oculomotor system should include the cover test, Hirschberg test, and Angle Kappa (all used for the determination of strabismus). The physiologic H test will rule out the presence of any paralytic extra-ocular muscle involvement and the near point of convergence test will diagnose the presence of a convergence insufficiency.

Table 2
Recommended Examination Techniques

Visual Acuity & Refractive Analysis	**Oculomotor**	**Ocular Health**
Teller Acuity Cards	Cover test	Hand held biomicroscopy
Broken Wheel	Hirschberg test	
Light House Cards	Angle Kappa	Hand held tonometer
Allen Cards	Bruckner Test	Tonopen
Illiterate E	Physiologic H	Goldmann
Dynamic retinoscopy		Burton Lamp
Cycloplegic retinoscopy		Monocular or binocular ophthalmoscopy
Placido's Disc		
Electrodiagnostic evaluation ERG, VEP, EOG		

Since 50% or more of the fra X individuals appear to have a significant amount of refractive error, it is important the optometrist or ophthalmologist have a high level of skill using objective assessment techniques. These techniques may include dynamic retinoscopy or the use of cycloplegics during this portion of the evaluation.

The assessment of ocular health should include a dilated fundus evaluation, tonometry (glaucoma work up), and a biomicroscopic assessment of the external eye. No known adverse side affects to mydriactics/cycloplegics (Cyclopentolate Hydrochloride 1%, Phenylephrine Hydrochloride 2.5%, Tropicamide 1%) have been reported in fra X patients. The use of these diagnostic pharmaceutical agents should be routine unless contraindicated for other reasons. Few ocular health anomalies have been reported for children with fra X, but it should be noted that during this conference both King et al. (1992) and Maino (Amin & Maino, 1992) reported seeing a child with a chorio-retinal atrophy of unknown etiology. Other diagnostic techniques to consider include the application of electrodiagnostic evaluations such as VEPs, ERGs, and EOGs (visually evoked potentials, electro-retinograms, electro-oculograms) (Seibert, 1993) and the use of various techniques associated with low vision assessments.

TREATMENT

The treatment of these associated eye and vision disorders should always start with the most appropriate correction. When properly applied, glasses will not only correct any refractive error present, but can eliminate induced ocular symptomology (diplopia, headache, eyestrain) and remediate both accommodative (focusing) and vergence (oculomotor) dysfunctions. Orthoptics, vision therapy, and/or surgery may be used to reduce or correct strabismus and to improve the visual acuity of an amblyopic eye (Maino, 1993).

SUMMARY

Every child diagnosed as having a developmental disability must have a complete eye examination no later than 6 months to 1 year of age. This evaluation should be conducted by a pediatric optometrist or ophthalmologist with an extensive background in working with individuals who have various handicapping conditions. It has been noted that, "all children and adults should have single, clear, comfortable, (binocular), and pathology-free vision if they are to achieve their highest potential. This becomes even more important for any person with a handicap" (Maino, 1990). Children with fra X must be considered at risk for the development of potentially sight threatening abnormalities. With early intervention many of these oculo-visual dysfunctions can be successfully treated when appropriately diagnosed.

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EYE SYMPOSIUM QUESTIONS & ANSWERS

Q: My child has numerous pairs of glasses he won't wear. Why doesn't he wear them?

A: There may be several reasons why a child won't wear glasses. One of the most commonly encountered reasons is that the child is farsighted (hyperopia). Children who are farsighted can often compensate for this refractive error by using their focusing system. Unfortunately, this means that the child is then forced to focus when looking far away (your focusing system should be relaxed when you look at a distance) and then he/she has to focus even more when looking up close. All this excessive focusing can cause headaches, blurred vision, diplopia (seeing double), eye strain, and even strabismus (esotropia or an inward turning of the eyes). Individuals who first receive glasses that correct for farsightedness should approach their use as if your doctor just gave you a new medicine to combat the flu. When you first take that medicine you probably still feel ill. As you use the medicine over the next several days, you start to feel better and better. Glasses that correct for farsightedness need to be used over several days or weeks before you may notice a change for the better. Given sufficient time, the "medicine" in the glasses should also start to work.

Children with fragile X syndrome may be hypersensitive to anything touching their face. You may want to work with your occupational therapist to decrease this hypersensitivity so your child can wear the glasses. With appropriate behavior modification techniques and encouragement, most children will be able to wear their glasses. On the other hand, don't drive yourself crazy trying to have your child wear the glasses! In some instances, it just takes a little more time.

Q: My family doctor never suggested that we schedule an eye examination for my child. Why not?

A: For all the publicity given to the topic of fragile X syndrome, and even with the hundreds of scientific articles written on the topic, few lay or professional people know much about the syndrome. Here's your chance to educate your doctor to the importance of all children having a complete eye evaluation. Do not hesitate to ask your doctor to call me (DM) if more information is needed (see address and phone number below).

Q: I've heard that "tinted lenses" may help my child learn to read. Is that true?

A: Although reading through "rose tinted glasses" may be the latest fad coming out of the west coast, there is currently no scientific evidence that these lenses help in any way. Helen Irlen devised a technique while preparing her masters' thesis she said would cure non-vision and/or non-language based reading problems. She called this the Scotopic Sensitivity Syndrome. At this time we do not recommend that you rush out to an Irlen Clinic for treatment. For additional information on this please see:

Solan, H. (1990) Learning disabilities. In Rosenbloom, A. A. & Morgan, M.W. (eds.) *Principles and Practice of Pediatric Optometry*, Philadelphia:Lippincott.

Scheiman M., Blaskey P., Ciner E., et al. (1990) Vision characteristics of individuals identified as Irlen Filter candidates. *Journal of American Optometric Association*. 61:600-605.

Q: My child had an eye examination at school. Is that adequate?

A: NO! Schools do not provide eye examinations only vision screenings. These screenings usually only assess distance visual acuity and will often miss other eye and vision problems. We recommend that every child routinely receive complete eye care on an annual basis from an optometrist or ophthalmologist.

Q: I've heard that children with fragile X syndrome have perceptual problems? What does that mean?

A: Individuals with visual-motor and/or vision perception abnormalities have difficulty with processing visual information. This means that all the information that is brought in using the eyes may get "mixed up" when the brain tries to figure out how to respond to it. Currently there are few studies looking into how children with fragile X process visual information. For more specific information concerning visual approaches to learning, see the Intervention section of this proceedings.

Q: How can I find an eye doctor that will work with my child?

A: The first thing we would recommend is that you check with other families who have children with handicaps. Word of mouth referral can work wonders! You may also want to call the state Optometry or Ophthalmology Association as well. Another resource would be to contact the local college of Optometry, children's hospital, or university in your area. I've (DM) given the National Fragile X Foundation a list of optometrists and ophthalmologists who work with children who have fra X or other handicapping conditions. Please call the Foundation for additional information.

Other resources include:

The College of Optometrists in Vision Development
P.O. Box 285
Chula Vista, CA 92012

Optometric Extension Program
Foundation, Inc.
2912 S. Daimler St.
Santa Ana, CA 92705

If all else fails never hesitate to call or write:

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Q: I'd like to find some place that is familiar with fra X and can provide most of my child's needs. How do I find such a place?

A: It's best to start by contacting the National Fragile X Foundation (1-800-688-8765). They keep a list of facilities that can provide the latest information for diagnosis and treatment. New clinics dedicated to serving families with children who have fra X are currently being established. One such clinic is now in the Chicago area. For additional information call: Mrs. Terry Treitman, Fragile X Association of America, 708-724-8626.

WORKSHOP SUMMARY: NEUROPSYCHOLOGICAL DEFICITS AND LEARNING STRATEGIES FOR FRAGILE X FEMALES

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This workshop was presented as part of the Third International Fragile X Conference on June 18, 1992. The specific aims addressed in the workshop were to 1) present up-to-date research findings concerning neuropsychological characteristics of females (children and adults) who carry the fragile X gene, 2) highlight the consistencies in research findings obtained from different research laboratories, and 3) synthesize across the researchers' findings to form a comprehensive picture of fragile X as a specific learning disability (in affected individuals) in order to suggest intervention strategies. Information concerning both research and educational applications was presented to emphasize the important connection between these two areas.

SUMMARY OF INDIVIDUAL PRESENTATIONS

Research findings from four laboratories were presented. Mazzocco presented information from an National Institutes of Health (NIH) research project currently in progress at The Children's Hospital in Denver. The focus of the study includes the neuropsychological, physical, and emotional characteristics of women, ages 18 to 45 years, who carry the fragile X gene and who score in the low-average range or higher on a standardized test of intellectual quotient (IQ). In this study, four specific domains of neuropsychological functioning were examined: 1) left hemisphere function, as measured by verbal memory and nonword reading tasks, 2) right hemisphere function, as measured by attentional, design memory, and visual-spatial tasks, 3) frontal lobe function, as measured by different problem solving and abstract reasoning tasks, and 4) long term memory functions, for both verbal and design memory. For this workshop, findings were reported for 16 women who carry the fragile X gene and who express the gene in $\geq 2\%$ of their cells (hereafter referred to as cytogenetically expressing women); 35 women who carry the fragile X gene but who do not express the gene cytogenetically (hereafter referred to as obligate carriers), and 57 control women who do not carry the fragile X gene, but who are related to a fragile X individual or have a child with a non-fragile X developmental disability.

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* Authors are listed in alphabetical order; Dr. Miezejeski, who also participated in this workshop, has a summary which appears as a separate paper in this issue of the proceedings.

The findings from this research support the notion of specific deficits among cytogenetically expressing fragile X women, *as a group*, relative to performance by control women and obligate carriers. The deficits seen are in skills from one of two domains: 1) attention and/or visual-spatial abilities, and 2) "executive function" skills thought to be monitored by the frontal lobes, and which are typically goal oriented; these skills include planning, mental flexibility in problem solving, the ability to simultaneously consider many pieces of information when problem solving, and abstract reasoning. The data also suggest that cytogenetically expressing women have more difficulty with novel tasks (and therefore at the onset of novel tasks) relative to control and obligate carrier women. In addition to these demonstrated difficulties, cytogenetically expressing women demonstrated strengths in verbal memory, both short and long term, as was measured with a story memory test.

It is important to note that the deficits reported here appear *not* to result from a global depression in all scores as a function of lower IQ among cytogenetically expressing women. The overall effects of IQ were statistically removed, and deficits were not seen in many areas such as reading and other language related skills. Thus a specific pattern seen in this study is of executive function and attention/visual-spatial difficulties and memory strengths among cytogenetically expressing women, but not among obligate carriers. (See Mazzocco et al., 1992, and Mazzocco et al., 1992b, for a more detailed description.)

Similar findings emerged from research conducted at the New York State Institute for Basic Research, presented by Hinton. Hinton et al. (1992) examined both the nature of the cognitive deficits observed in female carriers of fragile X and the effect of inheritance pattern on neuropsychological profiles. These researchers tested 20 women who inherited the fragile X gene from their fathers (paternal-inheritance group), 15 women who inherited the fragile X gene from their mothers (maternal-inheritance group), and 18 control women (mothers or sisters of persons with Down Syndrome or noncarrier sisters of males with fragile X). All of the women were 16 years or older and had IQ scores of 70 or better. The tests administered to these women were measures that emphasized memory, visual-spatial, and attentional skills. The results suggest the presence of cognitive deficits was related to inheritance pattern. Women in the paternal-inheritance group were no different from controls on any of the cognitive measures. However, women in the maternal inheritance group appeared to fall into one of two subgroups: one group was unaffected, and the other group manifested some cognitive deficits. The latter group consisted of approximately half of the maternal-inheritance group; each woman in this "affected" subgroup had an IQ score more than one standard deviation from average (i.e., 85 points or lower, whereas the average IQ score is 100). None of the women in the paternal-inheritance or the control groups scored in this range. Furthermore, all of the women with cytogenetically expressing cells were in the lower IQ maternal-inheritance group. Because this group appeared to be distinct from the other group of fragile X carriers on both genetic and cognitive measures, they were included as a separate group for the analyses described below.

The four groups (paternal-inheritance, the "affected" and non-affected maternal-inheritance, and the control groups) were then compared on specific neuropsychological measures with the overall effects of IQ statistically removed. Previous research has demonstrated that some fragile

X women have difficulty with a visual memory task in which they are asked to look at an abstract line drawing for ten seconds and then draw it from memory. The performance on this measure was examined more closely by measuring specific memory, visual spatial and attention functions. Results indicate that most fragile X women had no difficulty with any of these tests. However, the women in the *lower-IQ* maternal-inheritance group performed more poorly on attentional and abstract visual-spatial tests, yet no differently on memory tests, relative to the other fragile X women and the controls. Thus, these data are in agreement with those reported by other researchers: memory skills appear to be relative strengths and visual-spatial and attention skills appear to be relative weaknesses in cytogenetically expressing fragile X women.

Data on women were included in this workshop in order to examine the stability of neuropsychological profiles which emerge early in development. We were therefore interested in whether the data on women, as presented above, were consistent with data obtained on young girls who carry the fragile X gene. Borghgraef presented data regarding significant attentional problems, shyness and social anxiety in fragile X girls (Borghgraef et al., 1990).

Freund and Baumgardner presented data comparing 10 normal-IQ girls with fragile X to 7 non-fragile X, normal-IQ, control peers on 1) the Wechsler Intelligence Scale for Children - Revised (WISC-R), 2) an achievement test, and 3) a neuropsychological battery. The ages of the two groups were comparable: the average age among fragile X girls was 11.0 years (with a standard deviation of 3.8), and the average age among the control girls was 10.7 years (standard deviation = 2.7). The groups were matched on Verbal IQ in order to investigate questions on non-verbal-based abilities and executive function.

Using nonparametric statistics to examine group differences, the fragile X group showed lower Full Scale and Performance Scale IQs on the WISC-R. The WISC-R factor scores (which indicate specific categories of skills) were also analyzed. Although the two groups were comparable on the Verbal Comprehension Factor and the Performance Factor, the fragile X group was significantly depressed on the Freedom from Distractibility Factor, relative to the control group. Looking at the Verbal Comprehension/Freedom from Distractibility Factor Discrepancy for each girl, 7 of the 10 fragile X girls showed a statistically and clinically significant discrepancy, with a lower Distractibility Factor score relative to the Verbal Factor score. Only 1 of the 7 control girls showed such a discrepancy. Subtest deviations were also calculated for each girl to investigate patterns of relative cognitive strengths and weaknesses. The average deviation for each subtest for each group was derived. Comparisons of these average deviations replicated the previously established profile for fragile X weakness on the Arithmetic, Block Design, and Coding subtests. Overall, the WISC-R results suggest that the fragile X girls can be vulnerable to weaknesses in their ability to concentrate, ability to utilize short term memory--especially on tasks with little inherent structure--and in their ability to use visual-spatial skills.

Achievement test results using the Woodcock-Johnson-Revised showed that fragile X girls received lower scores on the math achievement subtest than did controls. However, reading achievement scores were comparable between the two groups.

Results from the neuropsychological battery were consistent with the WISC-R results, and helped define somewhat more precisely specific areas of deficit associated with the fragile X group. In terms of attention, results from a computerized continuous performance test (CPT) showed that fragile X girls were more impulsive and inattentive than were controls during the latter half of the task only, suggesting greater difficulty with attention maintenance. Additional executive function deficits were observed in fragile X girls when compared to controls. For example, fragile X girls show poorer category scores on the Wisconsin Card Sort Test, indicating difficulty in grasping abstract concepts and poor flexibility in shifting mental set. They also showed poor planning and organization skills for perception and recall of complex visual spatial relationships, which was reflected in lower organization scores from the Rey-Osterreith Complex Figure drawing. Deficits in visual-spatial orientation were evident from the lower judgement of line orientation scores and the lower copying inventory score from the Rey-Osterreith in fragile X girls relative to controls. Finally, fragile X girls demonstrated a relative strength in verbal learning and memory of unrelated words on the Selective Reminding Test for which their scores were comparable to controls.

In summary, these preliminary data suggest that girls with fragile X have deficits in both executive function and nonverbal, spatial awareness. These neurocognitive process deficits may underlie deficits observed in arithmetic and other tasks demanding mental visualization and attention. (See Baumgardner et al., 1992, and Freund & Reiss, 1991, for a more detailed description of related studies).

SUMMARY

One of the most compelling observations to be drawn from the workshop is the consistency across the four laboratories represented and across studies of girls and women. These consistencies point to attentional and problem solving difficulties which appear specific to fragile X as a learning disability, and to strengths in memory, particularly verbal memory. The studies of women suggest that such deficits appear to occur only in cytogenetically expressing females. Understanding the relation between factors such as cytogenetic status and inheritance pattern contribute toward information on prognosis and intervention. A description of fragile X as a learning disability and of appropriate intervention strategies follows in the Miezejeski & Hinton report.

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FRAGILE X LEARNING DISABILITY: NEUROBEHAVIORAL RESEARCH, DIAGNOSTIC MODELS, AND TREATMENT OPTIONS

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INTRODUCTION

Often, progress in scientific research seems to occur at a painfully slow pace. When the need for scientific progress is great, that slow pace can generate impatience and anxiety. In a recent article in *The New York Times* on the pace of progress in AIDS research, it was noted that even among those most in need of some scientific "shortcut" many recognize that in science there is frequently no quick fix (Altman, 1992). The present paper reviews the progress of behavioral science in its research of specific cognitive and behavior deficits seen in the fragile X syndrome, relates this progress to existing psychodiagnostic models and then proposes tentative treatment options.

RESEARCH

By genetic karyotype, half of the human population is described as 46,XX, or, as better known to most persons, the female sex. Approximately 1/700 of these females carries a defect called a fragile site at a specific location (band Xq27.3) on the distal end of the long arm of one of her X chromosomes (Brown, 1989). This defective X chromosome has become known as the fragile X or fra X, and the associated physical and psychological symptoms as fra X syndrome.

This paper will discuss only psychological symptoms. In particular, for reasons that stem from the status of neuropsychology as a clinical and scientific discipline, only less severe cases of fra X syndrome, called here fragile X learning disability (FXLD), will be described in any detail. Fra X causes mental retardation in about 1/1,200 males in the general population. Although fewer females are affected by fra X, and of those who are affected, few exhibit mental retardation, many more females than males present with FXLD. Of the 1/700 women who are carriers, about 35% have some mental impairment (Sherman et al., 1985). This is believed to be underestimated, however, because due to the milder effects of fra X in females than in males, many cases may go undocumented. Female carriers have a broader spectrum of phenotype than males, ranging from mental retardation to learning disabled to cognitively unimpaired. With the recent discovery that fra X syndrome is associated with a known mutated gene (Verkerk et al., 1991), it may soon be possible to identify in persons with fra X syndrome associations between molecular genetics and psychological development. Psychological evaluations of those who are not mentally retarded may offer insight into which specific functions may be more susceptible to deficits mediated by the mutated gene.

Cognitive Deficits

Because of the much less severe nature of psychological dysfunction seen in most females affected by fra X, it has been possible to evaluate in these persons the relative strengths and weaknesses of specific cognitive skills. Relative deficits have also been observed in persons with fra X mental retardation, though less frequently (Crowe & Hay, 1990; Hodapp et al., 1992; Miezejeski et al., 1987). Several studies of females with fra X have compared subtest scores on the Wechsler Intelligence Scales for patterns of relative deficit (Brainard et al., 1991; de von Flindt et al., 1991; Grigsby et al., 1987; Kemper et al., 1986; Miezejeski et al., 1986; Wolff et al., 1988). In the latter studies, mixed groups of cytogenetically positive (expressing the fragile site) and negative (not expressing) females were tested, and clear distinctions were not made between the groups. Nonetheless, with the exception of two studies in which nearly all the females were fra X negative (de von Flindt et al., 1991; Wolff et al., 1988), these studies consistently reported relative deficits on the arithmetic, digit span and block design subtests. Using the Stanford-Binet (4th Edition), Freund & Reiss (1991) noted deficits on quantitative skills, short-term memory for visually presented abstract stimuli, spatial visualization and visual-motor coordination, which are similar to those noted in studies using the Weschler scales.

Work in our laboratory and in others has suggested that levels of function on several neuropsychological variables differ between fra X carrier females who are fra X positive versus those who are fra X negative (e.g., see Baumgardner et al., these proceedings). Fragile X positive females have been shown to perform more poorly than controls on measures of attention, abstract visual-spatial and problem-solving skills. In contrast, carriers who are not cytogenetically expressing show no deficits on any of the cognitive skills tested. Of interest is that the fra X positive females appear to have circumscribed deficits; some skills, such as memory, are relative strengths for this group. Further, we have demonstrated a relationship between the presence of cognitive deficits and both the parental origin of the gene and its molecular structure (Hinton et al., 1992a).

Academic and Behavioral Deficits

Arithmetic Disorder As first reported by Hagerman and Smith (1983), and frequently replicated in subsequent studies of Wechsler intelligence test subscores, for fra X females as a group, performance on the arithmetic subtest is a relative weakness (Brainard et al., 1991; Grigsby et al., 1990; Kemper et al., 1986; Miezejeski et al., 1986; Steyaert et al., 1992). In addition, a more specific pattern of arithmetic deficit, spatial dyscalculia, has been observed in fra X females (Grigsby et al., 1990). Poor arithmetic performance by fra X females extends to measures of achievement, as first demonstrated by Miezejeski et al (1984), using the Wide Range Achievement Test-Revised. This finding has been confirmed recently by Mazzocco et al (1992), who, using the Peabody Individual Achievement Test-Revised, demonstrated that this relative arithmetic achievement deficit is limited to fra X positive females, which appears to account for the absence of a comparable relative deficit in the women evaluated by Wolff et al (1988). Relatively poorer performance on arithmetic achievement as compared to other measures of achievement has been noted in both fra X males who were also mentally retarded (Hodapp et al., 1992; Kemper et al., 1988) and in those who were not (Hagerman et al., 1985).

Attention Deficit and Social Withdrawal In both research and clinical reports, the most frequently noted behavioral difficulties associated with FXLD have been attention deficit disorders, whether with or without hyperactivity (Borghgraef et al., 1990; Hagerman & Smith, 1983; Lachiewicz, 1992), and social withdrawal (Borghgraef, et al., 1990; Cronister et al., 1991; Lachiewicz, 1992; Reiss et al., 1989; Uchida & Joyce, 1982), often referred to as "shyness." Reiss et al. (1989) observed increased psychological dysfunction primarily in fra X positive females who had inherited the fra X chromosome from their mothers. As described by them, social withdrawal appeared to be a central feature of the psychological disability seen in this group.

Mazzocco et al.(1992, also see Baumgardner et al., these proceedings) describe deficits on tasks of problem solving skills that rely on attentional processes. In addition, work from our lab has also demonstrated poor performance on tests believed to measure attentional skills. Affected fra X women showed deficits on both the Freedom from Distractibility Factor of the Wechsler Adult Intelligence Scale - Revised (Hinton et al., 1992a) and on a computer visual matching test (Hinton et al., 1992b).

Cognition and Behavior: A Neuropsychological Interface

In keeping with a traditional tenet of neuropsychology, investigators have attempted to hypothesize relatively circumscribed areas of cortical dysfunction that may be related to the patterns of functional deficit observed in FXLD. That is, unlike the diffuse deficiency of intelligence and cortical function presumed to occur in mental retardation, in FXLD, cortical dysfunction is assumed to be more limited, e.g., localized either to a neuroanatomically defined region, system, or type of neural tissue. The first attempt to hypothesize a localization of dysfunction in fra X syndrome (Miezejeski et al., 1984) suggested relatively greater right than left hemisphere involvement. Subsequently, this hypothesis has been proposed also by others (Crowe & Hay, 1990). The most recent evidence from neuroimaging studies offers tentative support for this hypothesis (Murphy et al., 1992; Murphy et al., 1991; Reiss, 1992). Nonetheless, given other findings suggesting deficits localized to other regions, e.g., the frontal lobes (Mazzocco et al., 1992; Mazzocco et al., 1992b), the dominant parietal lobe (Grigsby et al., 1987), and even more specifically the angular gyrus of the dominant hemisphere (Grigsby et al., 1990), conclusions about localized dysfunction are yet to be resolved. Some of these suggested cerebral correlates of the functional deficits seen in FXLD have a direct bearing on the following discussion of diagnostic models.

DIAGNOSTIC MODELS

As a result of research efforts over the past ten years, scientists and clinicians have begun to identify some of the major psychological symptoms of FXLD. Even prior to the initiation of that research, neuroscientists had described other cognitive/behavioral disorders with similar neuropsychological symptomatology. It is suggested here that some of these disorders can serve well as diagnostic models for further study of FXLD and for formulating treatment approaches.

Three possible diagnostic models for FXLD are discussed: 1) Attention deficit disorder (American Psychiatric Association, 1987), 2) Nonverbal learning disability (NLD) (Myklebust, 1975; Rourke, 1987), and 3) Social learning disability (SLD) (Weintraub & Mesulam, 1983).

FXLD as Attention Deficit Disorder

Attention deficit disorder, both with and without hyperactivity is frequently observed in persons with FXLD. Further, "academic underachievement is characteristic of most children with this disorder" and "motor-perceptual dysfunctions ... may be present." (American Psychiatric Association, 1987). Thus, attention deficit disorder appears to include most of the features of FXLD identified in the studies described. Moreover, research suggesting frontal lobe dysfunction in FXLD (Mazzocco et al., 1992b,) lends support to the presence in FXLD of a disinhibition of some of the cerebral mechanisms involved in managing attentional processes (Luria, 1966).

Given the presence of frequently noted symptoms that are not easily explained as due to attention deficit disorder, however, the symptomatology of attention deficit disorder does not provide a comprehensive diagnostic model for FXLD. For example, an equally prevalent symptom of FXLD, passive social withdrawal, is rarely noted as characteristic of attention deficit disorder, but rather is quite the opposite of many of the social interactions typically seen in children with attention deficit disorder. Social withdrawal may occur as a child with attention deficit hyperactivity disorder reaches adulthood, but it is usually any thing but passive. That is, a not uncommon outcome in adulthood is antisocial personality disorder (American Psychiatric Association, 1987). In that respect, attention deficit hyperactivity disorder meets some of the criteria of the more inclusive diagnostic model of nonverbal learning disability. For a more thorough discourse on attention deficit disorder with a case description of a six year old male with FXLD and attention deficit disorder, see Keogh (these proceedings).

FXLD as Nonverbal Learning Disability

Nonverbal learning disability (NLD) is a term used to identify a characteristic neuropsychological profile observed most frequently among individuals who possess the central trait of academic performance that is marked by arithmetic disability in the presence of adequate reading and spelling. These individuals also exhibit perceptual-motor and social deficits, both of which worsen with age. Rourke, who has devoted much effort to developing neuropsychological models of learning disabilities (Rourke, 1978, 1982) has investigated NLD extensively (Rourke et al., 1986; Strang & Rourke, 1985a, 1985b), including the potential impact of such learning disability on socioemotional development (Rourke, 1988).

A condensed version of the nine defining traits of NLD described by Rourke (1987), are presented in Table 1 with citations of reports that describe the status of these traits in FXLD. As that tabulation should suggest, the similarities of FXLD to NLD are numerous. Although there exist currently several critical gaps in the data on FXLD, there is at least the suggestion of some divergence of features. One of the more striking differences in features has to do with the dimension of psychomotor coordination.

Table 1
FXLD as Nonverbal Learning Disability

	Defining Traits of NLD	Presentation in FXLD
(1)	Tactile-perceptual deficits on left and right side, more on the left than on the right	Not evaluated
(2)	Psychomotor coordination deficits: more on left side than right side	Without reference to laterality: Grigsby et al. (1990); Hagerman et al. (1985)
(3)	Visual-spatial-organizational deficits	Cianchetti et al (1991); Grigsby et al (1990); Hinton, et al. (1992b); Miezejeski et al. (1984, 1986)
(4)	Deficits in nonverbal problem solving, including using information feedback in novel or complex settings	Mazzocco et al. (1992b)
(5)	Very well developed rote verbal skills, including extremely well developed rote verbal memory	Borghgraef et al. (1990)
(6)	Extreme difficulty adapting to novel or complex situations, with overreliance on mechanical., rote behaviors	Poor learning of novel task: Mazzocco et al. (1992b)
(7)	Relative to reading and spelling, very poor arithmetic	Mazzocco et al. (1992b); Miezejeski et al. (1984, 1986)
(8)	Reliance on language, for social relating, learning, and relief from anxiety	Not evaluated
(9)	Interpersonal difficulties and socioemotional disturbances, which increases with age	Cronister et al. (1991); Lachiewicz (1992); Reiss et al (1989)

As described in his many papers on the subject, Rourke's description of the reliability of the various traits of NLD, suggests that in the hierarchy of the traits, the presence of psychomotor coordination deficits is second only to relatively greater arithmetic deficit (Rourke, 1982, 1987, Rourke et al., 1986; Strang & Rourke, 1985a, 1985b). Studies of persons with FXLD suggest that, at least for adult females, psychomotor coordination deficits are far from being a reliable trait, often not being present despite evidence of possible visual-spatial-organizational deficits (Hinton et al., 1992b; Miezejeski et al., 1986). In addition, directly opposite to reported increased psychomotor coordination deficit with increasing age in NLD (Rourke et al., 1986) is the observation of an association between improved performance and increasing age in persons with FXLD (Grigsby et al., 1990). Further, though there is no research data to suggest that the socioemotional difficulties of FXLD do not intensify with increasing age, clinical observations by one of these authors (C.M.M.) suggest that, unlike what has been described for NLD (Rourke et al., 1986), this is not the case in FXLD.

Another interesting characteristic of NLD similar to findings in FXLD is reliance on language to learn. Although visual-spatial deficits have been repeatedly demonstrated in FXLD (Cianchetti et al., 1991; Grigsby et al., 1990; Hinton et al.,1992b; Miezejeski et al., 1984, 1986) the presence of such deficits may be circumscribed to abstract visual stimuli without verbal cues. Freund & Reiss (1991) report deficits in visually presented abstract stimuli, and work from both our lab and theirs found impaired performance on the Benton Line Orientation Task (see Baumgardner et al., these proceedings) which involves nonverbal perception of line position in space. In contrast to that, we have shown that on a visual-spatial constructional task where pictures of familiar things are cut up and must be mentally put together to determine what the picture is (the Hooper Visual Organization Test), FXLD subjects show no impairment (Hinton et al., 1992b). We believe this is due, in part, to the presence of salient features of the stimuli that subjects can name, (e.g., in a cut-up picture of a fish, the eye and the fin are clearly visible in the parts). Thus, in FXLD (as in NLD) verbal cues may be used as strategies for solving visual-spatial problems, although more research must be done to look closely at this.

Although there are many similarities between NLD and social learning disability as described below, we prefer to treat them as separate diagnostic models. This preference is based on what appears to be a difference between them in terms of the relative importance of defining characteristics. While this difference may prove to be an artifact of differences in research interests and approaches taken by the different scientific groups studying these disorders, we suspect that these are empirically divergent entities. In particular, their divergence rests at least in part on a difference in the severity and quality of the socioemotional characteristics reported. In NLD, there appears to be a more severe presentation of behavioral disturbance associated with the reported social deficits than is described for SLD. Nonetheless, these two diagnostic models of learning disability appear to be related along this dimension of social disability, as well as by an emphasis in reports on both of these learning disabilities of possible right hemisphere dysfunction.

FXLD as Social Learning Disability

Social learning disability (SLD) is the term used here to describe what has been called social-emotional learning disabilities (Denckla, 1983) and developmental learning disabilities of the right hemisphere (Weintraub & Mesulam, 1983). Placing considerable emphasis on the involvement of the right cerebral hemisphere, Weintraub & Mesulam (1983) have described its key features as including emotional/interpersonal difficulties, shyness, visuospatial disturbances, and inadequate paralinguistic communication skills (Weintraub et al., 1981).

In Table 2, the defining traits of SLD, as described by Weintraub and Mesulam (1983), are presented with citations of reports that have described the status of these traits in FXLD.

As Weintraub and Mesulam (1983) have described it, SLD is primarily a disorder that includes socioemotional traits that are of a passive avoidant nature. In NLD, the socioemotional symptomatology is much more virulent, i.e., there are serious disturbances of social relating much more like the antisocial personality disorder that is often an adult outcome of attention deficit hyperactivity disorder (American Psychiatric Association, 1987). The more passive presentation of socioemotional deficits as seen in SLD appears to be much more similar to that seen in FXLD, as described in studies referenced in table 2.

Table 2
FXLD as Social Learning Disability

Defining Traits of SLD	Presentation in FXLD
Emotional and interpersonal difficulties	Lachiewicz (1992); Reiss et al. (1989)
Shyness	Borghgraef et al. (1990); Cronister et al. (1991); Hagerman & Sobesky (1989); Uchida & Joyce (1982)
Visuospatial disturbances	Cianchetti et al. (1991); Grigsby et al. (1990); Hinton, et al. (1992); Miezejeski et al. (1984, 1986)
Inadequate paralinguistic communication skills:	
Avoidance of eye contact	Reduced eye contact: Hagerman & Sobesky (1989) No difference in eye contact: Cronister et al. (1991)
No gesturing while speaking	Not evaluated
Lack of prosody in speech	Not evaluated

TREATMENT OPTIONS

Attention Deficit Disorder

Stimulant medication has been the most frequently used, and often the most effective treatment for attention deficit disorder. A double blind study of this approach with children who have fra X syndrome indicated improved attention span (Hagerman et al., 1988). Of the 15 children in this study, however, only 3 had IQs greater than 70, and none were either of normal intelligence or could be said to present with FXLD, as described here. Thus, the efficacy of stimulant medication for treatment of FXLD is yet to be determined.

Other treatment options for attention deficit are discussed in these proceedings by Keogh (1992) with a presentation of the result of neuropsychological training of attention skills in one FXLD boy.

Arithmetic Deficits

Strang and Rourke (1985) have offered a teaching strategy for the improvement of arithmetic skills in children who exhibit the neuropsychological characteristics of NLD. The arithmetic deficits observed in FXLD have neuropsychological bases very similar to those seen in persons with NLD. The following is an abridged version of the strategies described in Strang and Rourke (1985), modified only slightly here as a teaching strategy recommended for persons with FXLD:

1. Begin with a simple calculation that has been problematic for the child.
2. Describe the calculation to the child in words.
3. Present verbally the steps involved in the mathematical operation in complete detail.
4. Have the child give a systematic account of these steps.
5. Have the child write out the rules for this particular operation. Check their accuracy, and allow the child to use this "recipe" as long as is needed.
6. When and only when the verbal steps have been mastered, it is then time to acquaint him/her with the visual aspects of the operation. Have the child direct the teacher through a sample calculation with his/her verbal rules.
7. Provide concrete (physical) aids (e.g., number boards, etc.) to introduce the underlying mathematical concept.
8. Next, have him/her practice using graph paper to minimize problems with spatial orientation.
9. If needed, color-code the graph paper to facilitate left-right discrimination. Draw a vertical red line on the left side of the page or even to the left of each arithmetic problem and a horizontal green line at the top of the page or even above each arithmetic problem. Have the child orient to the color-coded lines, rather than to left or right and top or bottom. For example, in arithmetic problems requiring the carrying of numbers from right to left columns, tell the child to carry the number to the next column toward the red line. With fractions, refer to the numerator as the number closer to the green line, rather than as the top number or the number above the line. This may help to reduce errors due to spatial dyscalculia.
10. Encourage the child to read each question aloud before beginning any mathematical operation.
11. Have the child use a calculator to check the accuracy of his/her answers.
12. Record all of the child's arithmetic errors and task-analyze these errors.

13. Promote generalization of mathematical procedures by relating the operation to some practical aspect of everyday life.

For a more detailed description of the above strategies, all of which are appropriate for use with children with FXLD, the reader should see the original presentation by Strang and Rourke (1985).

Social Learning Deficits

It has been suggested here that SLD, as seen in FXLD, may be associated with more general neuropsychological deficits of visual spatial and attentional processes. Based on our description above of FXLD as SLD, we propose the deficits seen in FXLD that are of a visual spatial nature may contribute directly to the development of secondary socioemotional symptoms. Persons with FXLD may be unable to comprehend many of the nonverbal cues that are an integral part of social communication. Thus, we suggest persons with FXLD would benefit from a program of therapeutic intervention that focuses on increasing their recognition and use of these nonverbal cues. The nonverbal cues of social communication are largely facial expressions and body language.

Minskoff (1980) published a comprehensive program for teaching social competence, one part of which emphasizes strategies for learning to interpret and use of facial expressions and body language, i.e., gestures and postures appropriate for social communication. The program involves specific teaching activity sequences for developing skills to discriminate, understand, and use body language cues appropriate to a particular communication situation. What follows is a much abbreviated version of some of her recommendations (Minskoff, 1980).

With some of the specific examples used by Minskoff, the following are four areas of instruction she indicates as necessary for developing social competence.

1) Students must be trained to discriminate critical visual and auditory social cues in the behavior of others and in themselves. For example, the student is taught facial features like the O-shaped mouth for surprise.
2) Development of an understanding of the meaning of these cues by verbally describing specific communication situations in which the cues occur is necessary.
3) Students need to be trained to recognize the expression of specific motor and oral cues; that is, building the specific social responses into the student's response repertoires. One method is to use role playing. For example, "in a skit about a lunch-table conversation, have the students relate one person's description of his dog vomiting to another person's facial expression" (Minskoff, 1980).
4) Train the students to discriminate negative, nonverbal social cues in persons with whom they are interacting, to relate these cues to specific responses they have made, and then to modify such responses in future situations of this nature. For example, "in a skit in which a boy continuously interrupts the teacher with questions while she is giving directions to the total group, have students relate the teacher's repeated swift, curt gestures for 'go away' to the objective of the communication situation that is to convey information to the group" (Minskoff, 1980).

ACKNOWLEDGMENTS

The authors wish to acknowledge Deena Demonte and Caroline Hayes for assisting with the preparation of this manuscript.

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THE EMOTIONAL PHENOTYPE IN MILDLY AFFECTED CARRIERS

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The following information is taken from a five-year study of the neurocognitive, emotional and genetic features of fragile X females. This study is funded through the National Institutes of Mental Health grant #MH45916. These results are based on the first and second years of data collection. The number of individuals in each of our subgroups is still rather small. Hence, the patterns described should be considered tentative.

Before presenting our findings we will review the model of emotional functioning we are using to guide our understanding of the emotional phenotype in mildly affected carriers. We believe there are three major causes of emotional difficulties: genes, stress and developmental experiences. We will address each of these briefly.

The FMR-1 gene is considered to be the cause of the various cognitive differences, such as lower IQ scores or specific learning problems associated with fragile X. The FMR-1 gene could affect emotional behavior either directly through, for example, an alteration in neurotransmitter functioning, or indirectly, as for example, in the case of individuals who experience emotional problems secondary to neurological deficits.

Another pattern of genetic involvement includes background genetic factors which, often through the interaction of a number of genes, could lead to patterns of learning strengths and weaknesses, attentional problems, or temperamentally-based personality styles. Because of the success of scientists in identifying the fragile X gene and in beginning to understand at a molecular level what goes wrong in that gene, for example, the length of CGG repeat sequences, the genetic influences are particularly exciting at the moment. However, we believe there are other causes of emotional difficulty which are also important to recognize.

Stress is a broad based term covering a host of environmentally based factors which can affect an individual and a family system. Stress can be thought of as *situational* when it appears to be time-limited, as in the loss of a job; or as *chronic*, such as in the case of a medical problem that cannot be reversed. In actuality, it is often difficult to tell the two apart. Further, the degree of impact of a stressor on an individual is often idiosyncratic and unique to that person and their life circumstances. Stress affects emotional functioning in that it places an additional burden on an individual's ability to cope with life's demands. Emotional symptoms such as depression or anxiety can arise when an individual's coping skills have been overwhelmed by environmental pressures.

Several types of stress seem pertinent to fragile X carriers and their families. Raising a child is a difficult and rewarding task. But children with special needs, especially when those needs are not recognized or understood by others, is demanding. Over time, it can take its toll on parents' coping resources.

Problems associated with child-rearing underline the importance of two other situational factors: economic resources and social support. Economic advantage can enable parents to expand their resources by obtaining help and respite. Social support can supplement parents' coping skills and emotional resiliency so they are more able to deal with stress.

Finally, developmental experiences, often within one's family of origin, can also have a negative impact on a person's emotional functioning as an adult. These experiences range from a lack, or paucity, of basic emotional nutrients important for healthy growth to obviously destructive experiences such as child abuse. Parents who themselves lack critical social skills or who are coping with difficult psychological problems, such as depression, may have difficulty giving to their children the basic experiences critical for their children to become well-functioning adults. Ironically, such deficiencies may not become apparent until their children reach adulthood and become parents or spouses themselves.

To summarize, emotional difficulties in adulthood can arise from any of three sources: genes, stress, or developmental experiences. To understand the emotional phenotype of fragile X carriers, we must eventually look at all three of these factors.

In presenting research findings, we are usually talking about groups of individuals. Our results address a mythical "average" person in those groups of individuals. Our view is that specific human beings are much more complex than such groups. Hence, it may be a rather rare occurrence when only one factor is solely responsible for a particular pattern of emotional behavior. More likely is the possibility that individual emotional phenotypes are the result of an interaction, over time, of all three of these factors, as well as perhaps others we have not identified.

To return now to our study, women, between the ages of 18 and 45, whose IQ was 70 or higher, were recruited to participate. These women were paid for their participation. They were assigned to one of three categories (Table 1).

Table 1
Grouping Criteria

Group	Criteria	n
1	Controls:	48
	(a) Fra X Negative - many with children with developmental problems	(34)
	(b) Fra X Negative - cytogenetically and DNA; from a fra X family	(14)
2	Fra X Heterozygotes - cytogenetically negative; DNA positive	23
3	Fra X Heterozygotes - cytogenetically positive; fragility >3%. DNA positive	13

Category one consists of two control groups: in the first are women who are negative for fragile X, many of them are mothers of children with developmental problems, in the second are women who are negative for fragile X and who grew up in families with a fragile X member, generally a parent and/or sibling. Category two consists of women who are cytogenetically negative, but are DNA positive. Often they are mothers who have children who are fragile X positive. Category three consists of women who are cytogenetically positive. Their expression rate is greater than 3%. We selected this cut-off level for positive fragility to eliminate women who are cytogenetically positive but DNA negative. The average expression rate of Group 3 is 12% and the range is 3.5% to 35%.

The average age of Group 1 is 31 years, of Group 2, 34 years, and of Group 3, 30 years. As you can see, the numbers are still somewhat low for Categories 2 and 3 and so again, these results should be considered preliminary.

All women are administered the Wechsler Adult Intelligence Scale - Revised (WAIS-R) (Wechsler, 1981) as part of the neurocognitive battery. As part of the emotional battery, they completed the Schedule for Affective Disorders and Schizophrenia - Lifetime version (SADS-L) (Endicott and Spencer, 1978), the Structured Interview for Schizotypy (SIS) (Kendler et. al., 1989), and the Minnesota Multiphasic Personality Inventory-2 (MMPI-2) (Hathaway and McKinley, 1989). The same interviewer, who was blind to the cytogenetic status of the subjects, administered both the SADS-L and the SIS.

The WAIS-R is an individually administered intelligence test with a mean of 100 and a standard deviation at 15. Full scale IQs are presented in Table 2.

Table 2
Full Scale IQ Data

Group	Mean*	Deviation	Minimum	Maximum
Sample	101.5	14.8	72	139
1	106.9	14.1	76	139
2	99.0	10.2	84	122
3	86.2	12.5	72	117

*Group 3 < 1,2

As can be seen, while the overall sample mean is close to the norms (mean of 101 and standard deviation of 14.8), the groups do vary somewhat. Group 1, with a mean of 106, and Group 2, with a mean of 99, are significantly higher than group 3, which has a mean score of 86. Despite these mean differences, the ranges of the three groups, as seen by comparing the minimum and maximum scores, are fairly similar. The finding that carriers who express cytogenetically have lower IQ scores has been reported previously (Reiss et al., 1988). To evaluate the effects of IQ on emotional variables, analyses were performed which revealed group differences with the effects of IQ removed. That is, such effects were controlled.

The SADS-L is a semi-structured interview designed to yield information to make DSM-III-R diagnoses of affective disorders such as Major Depression and Generalized Anxiety Disorder, as well as schizophrenia spectrum disorders. SADS-L data is presented in percentage form in Table 3.

Table 3
Percentage of Subjects for Selected SADS-L Variables

Group	Counseling > 3 Months	Psychotropic Medications	Major Depression	Dysthymia	ADHD Past
1	28%	19%	33%	29	17%
2	13%	30%	30%	22%	13%
3	23%	31%	46%	31%	8%

As can be seen, there are no statistically significant differences between the three groups. There are some possible trends, however, that are of interest, in terms of seeking help for dealing with emotional difficulties. Group 2 women are twice as likely to use medications (30%) than they are to use counseling of more than 3 months duration (13%). The pattern is somewhat similar for Group 3 women, though not as pronounced. Group 1 women are more likely to use therapy than medications.

In considering depression, Group 1 and Group 2 are similar in the incidence of Major Depressive Disorder (33% and 30%, respectively), while Group 3 appears to have a somewhat higher incidence at 46%. Group 2 is somewhat low in incidence of dysthymia compared to Groups 1 and 3, while Group 1 is somewhat higher in the incidence of childhood attention deficit disorder. This higher incidence of attentional problems is consistent with the fact that a number of control group women are mothers of children with Attention Deficit Hyperactivity Disorder (ADHD).

The MMPI-2 is a self-report personality inventory designed to yield scores in a variety of personality and psychopathology-related dimensions. MMPI-2 raw scores are T-scores with a mean of 50 and standard deviation of 10. A score of 65 or higher is considered to lie in the top 8% of the distribution and to indicate a more serious problem.

On the MMPI-2 (Table 4), Group 3 is significantly higher than Groups 1 and 2 on the L or Lie Scale. Such an elevation suggests that this group may tend to deny problems or to present themselves in an overly conforming manner. Interestingly, this difference was not significant when the effects of IQ were covaried. Thus, it may be that individuals with fewer intellectual resources are more likely to utilize this coping strategy, regardless of group status. Finding a tendency for carriers who are cytogenetically positive perhaps to deny difficulties is consistent

Table 4
Mean Values of MMPI-2 Scales by Group

Group	L*	D*	Sc	Pt	Si	Anx***
1	53	55	50	53	54	55
2	54	56	48	54	53	53
3	62	49	50	48	50	51

* Group 3 > 1
** Group 3 < 1,2 (FSIQ Covaried)
*** Group 3 < 1 (FSIQ Covaried)

with some of our clinical experiences. We do not believe that individuals in this group deliberately lied but rather they seem to focus very much on the here and now in describing themselves, as if they are wearing blinders that interfere with integrating past difficulties with a current conception of self. This focus suggests something of an "out of sight, out of mind" approach toward dealing with problems.

As can be seen from the rest of these clinical scales, Group 3 women report consistently the lowest scores, "D" refers to Scale 2 which reflects symptomatology consistent with depression; "Sc" to scale 8 which are schizophrenia spectrum symptoms; "Pt" to scale 7 which are symptoms of anxiety and worry; "Si" to scale 0 which is social introversion; "Anx" is one of the new content scales developed by Butcher and his colleagues (1989) and reflects general symptoms of anxiety. When IQ is covaried, Group 3 is significantly lower on the D scale than Groups 1 and 2. Again, controlling for IQ, Group 3 is significantly lower than Group 1 on the Anx scale. These findings are somewhat surprising, because we would expect that individuals who have a lower IQ may have fewer resources to use in coping with the problems of life. Hence, they may be more likely to have difficulties or to become overwhelmed. Table 5, which shows correlations between IQ and three MMPI-2 scales, L, D, and Anx, suggests that this is generally the case, with the exception of Group 3. There are negative correlations between IQ and the D scale and Anx scale in Groups 1 and 2, suggesting that more cognitively able individuals report fewer symptoms of depression and anxiety. However, in Group 3 there is a small, non-significant, positive correlation between IQ and the D scale and virtually no relationship between IQ and the Anx scale. Perhaps brighter individuals in Group 3 are not so likely to use denial as are other individuals in the group, or greater intellectual ability is not as much of a protective factor in that group.

Table 5
Correlations of IQ With MMPI Scale by Group

MMPI Scale	Overall	Within Groups 1	2	3
L	-.33	-.22	-.28	-.13
D	-.17	-.38*	-.43**	.19
Anx	-.18	-.37*	-.30	.02

* $p < .01$
** $p < .05$

The SIS is a structured interview designed to obtain information on 16 aspects of schizotypal behavior. In addition, the interviewer rates each subject on a number of dimensions, based upon their behavior during the entire interview process (including the SADS-L). On the SIS, lower numbers indicate greater problems. We have selected two sets of variables to review (Table 6): the first has to do with social behavior in childhood (Childhood isolation) and adulthood (Current isolation) as well as adult shyness (Introversion); the second reflects the interviewer's ratings of subjects on three dimensions related to schizotypy. Those dimensions are affect, thinking and oddness.

Table 6
Mean Values of SIS Variables by Group

Group	Childhood Isolation	Current Isolation	Introversion	Interviewer's Ratings Affect*	Thinking**	Oddness**
1	4.4	5.1	4.8	4.1	4.5	4.5
2	3.8	5.1	4.8	4.2	4.4	4.4
3	3.5	4.6	4.9	3.5	2.9	3.2

* Group 3 < 2 (FSIQ Covaried)
** Group 3 < 1,2 (FSIQ Covaried)

As can be seen, there are no significant differences between the three groups on the social variables although there is a tendency for Group 3 to be more isolated than Groups 1 and 2, both in childhood and adulthood. There are significant differences on all of the interviewer ratings (the interviewer was blinded to group status). Group 3 is lower than Group 2 on the affect dimension, indicating less well-modulated and less appropriate expression of feeling than Group

2. Group 3 is lower than Groups 1 and 2 on the thinking dimension suggesting behaviors such as poor topic maintenance and more tangential communication than both Groups 1 and 2. Group 3 is lower on the oddness rating which points to behaviors such as gaze aversion and social distance. Such a pattern suggests that Group 3 carriers are more likely to display behaviors consistent with some schizotypal symptoms than are individuals in the other two groups.

Overall, these findings are consistent with those reported earlier by Reiss and his colleagues in several papers. Our rates of major depression are similar to those reported by Freund et al (1992), as is the finding that cytogenetically positive carriers may suffer from affective disorder to an extent as great or greater than cytogenetically negative carriers. In addition, we have documented that positive carriers may tend, in some circumstances, to minimize problems they experience.

SUMMARY

It appears some cytogenetically positive carriers are likely to have problems with depression and/or to display schizotypal-spectrum behaviors. Likewise, they may minimize problems and possibly be more socially isolated as children and adults. These results, in conjunction with those of other researchers, suggest the beginnings of an emotional phenotype for fragile X carriers.

The precise relationship of this phenotype to the fragile X gene is still unclear. To take a single example, depression could be the result of a direct alteration of neurotransmitter action by the fragile X gene, or depression may be related to difficulties in the modulation of affect which are secondary to neurocognitive deficits, particularly the frontal deficits reported by Mazzocco, et. al. (1992a and 1992b).

We found no statistically significant differences between cytogenetically negative carriers and controls. However, this does not mean that some do not experience difficulties. Rather, their difficulties, like those of controls, may be related to chronic stressors, such as raising a child with developmental problems, or to difficulties associated with their families of origin. Such experiences may or may not interact with gene effects to lead to emotional difficulties. At the present time, we cannot say.

Our clinical experience has led us to conclude that a life-span approach is important in considering psychological problems and fragile X. Difficulties related to child-rearing, marital relations, or estrogen deficiencies, for example, could lead to problems for women who had done rather well in managing the challenges of life until the time of one of these added difficulties.

Because of these challenges, we support the use of various intervention strategies for all carriers. There are a variety of biological and non-biological intervention techniques which have proven helpful in dealing with depression, anxiety and social relationship issues. The use of the treatment, in our mind, does not constitute the failure of an individual to manage their life, but rather an adaptive acknowledgement that at times help is needed. Such help can not only serve to improve difficulties that have already manifested themselves, but it can also provide protection against the development of future, more severe problems.

ACKNOWLEDGEMENTS

This research was supported by a grant from NIMH #MH45916

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THE LANGUAGE SYSTEM OF MALES WITH FRAGILE X SYNDROME

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INTRODUCTION

Language is a system of symbols (either spoken or acted) which when combined in specific ways, enables one member of the community to convey a meaningful thought to another member of the same community. The ability to give and take verbal information can be thought of as a hierarchical, intertwined combination of several competencies. The superordinate level consists of receptive and productive competence. Receptive competence is the ability to receive and understand the speech/language signal and act upon the command or request in a way that suggests understanding. Productive competence is the ability to produce meaningful, intelligible sentences that say what the speaker means to say. Subordinate to *both* receptive and productive skills are:

(a) **syntactic competence**, which is the ability to put words together to form questions, statements, negations, phrases, clauses and sentences;

(b) **semantic competence**, which is the knowledge of word meanings (e.g., that the word "girl" entails the features of female, two eyes, two legs, the ability to walk, etc.) and world knowledge (e.g.,the knowledge that mountains do not fly, or that trees do not talk), and

(c) **pragmatic competence**, which includes, among many aspects of extralinguistic competence, the knowledge of how to listen, how to carry on a conversation, how to initiate conversations, how and when to ask questions, how and when to answer questions, how to maintain eye contact so that the person with whom you are talking knows that you are listening and understanding.

In order to be a proficient user of language, the individual must have strengths in all of the above areas. However, during the development of language mastery, both normally and non-normally developing children present with both strengths and weaknesses in these language areas. For instance, a child could manifest receptive syntactic and semantic competence by understanding most of what is said to him (e.g., by carrying out the commands) while not being able to produce meaningful sentences which in turn would signify a productive deficit. Further, a child could be able to imitate everything that he hears, thereby exhibiting a form of productive syntactic competence, while not understanding much of what is said to him, suggesting deficient receptive semantic ability.

It is important to understand the distinctions among linguistic competencies described above, in order to begin to understand the distinctions made in this article on the possible causes of the production of language deviance by males with fragile X syndrome (fra X). Deviance here is defined as a behavior (verbal or nonverbal) that is (1) not produced by the non-delayed individual or (2) not produced to the same extent or within the same environments in the non-delayed individual. We will now turn to a discussion of the language of males with fra X.

One of the most characteristic language deviances produced by males with fra X is perseverative language (repeating words, phrases or topics of conversations over and over again). There are several possible explanations for the production of perseverative language: (1) an inability to locate the next word or thought in long term memory thus continuing with the statement or conversation; (2) an impulsivity due to stating what is on one's mind without adhering to conventional conversational structure; (3) an anxiety disorder perhaps exacerbated by an aversion to eye contact; and (4) a lack of familiarity with the acceptable word order patterns of one's language resulting in perseverating on the known while searching for the correct pattern to facilitate the production of the thought. These possible explanations are not mutually exclusive, and any explanation or combination of explanations begins to account for the characteristic language that is present in males with fra X. Most of the research presented below was motivated by the search for the causes of this perseverative language in males with fra X.

The areas discussed in this chapter are the results of investigations into syntactic and semantic competence. We have investigated productive syntactic competence and receptive and productive semantic competence. We will also briefly touch on some preliminary findings of the relationship between social anxiety and the production of perseverative language. First, however, we will describe the initial studies in perseverative language performed in our laboratories (Sudhalter et al., 1990).

PERSEVERATIVE LANGUAGE

Verbal productions of males with fra X, autism and Down Syndrome (DS) of comparable chronological and communication ages as well as adaptive behavior score on the Vineland Adaptive Behavior Scale (Sparrow et al., 1984) were divided into three mutually exclusive and all-inclusive pragmatic production categories: (1) direct response; (2) initiation; and (3) topic maintenance. These pragmatic conversational categories were analyzed for the presence or absence of deviant repetitive language. Deviant repetitive language was defined as perseveration, which could be: (a) phrasal (e.g., "I can't, I can't"); (b) sentential (e.g., "I want to go home. I want to go home." or "Bob Hope holds the microphone this way. Bob Hope holds the microphone this way."); and (c) topic (e.g., talking inappropriately and incessantly about the cartoon "Tom and Jerry," or puzzles or a particular television program). In addition to phrasal, sentential and topic perseveration, jargoning, echolalia and affirming by repetition were also scored as deviant repetitive language.

Results of our analyses suggested: (1) males with fra X present with significantly more deviant language than their matched peers with DS within all conversational contexts; (2) males with autism present with significantly more deviant repetitive language than their matched peers with either fra X or DS; (3) counter-intuitively, there is no difference between the percentage of deviant language produced by males when interacting with their parent caretaker or with an unfamiliar person; lastly (4) the most prevalent deviant language act produced by males with fra

X is perseverative language, while the most prevalent type of deviant speech produced by males with autism is echolalia.

Corroborating the above findings that the most prevalent language deviance males with fra X produce is perseverative language, are results of questionnaires filled out by parents of fra X males, males with retardation and males with autism. Parents delineated those behaviors that best described their children (Hagerman et al., 1991; Lachiewicz, 1989). One of the behaviors that best discriminated males with autism and retardation from males with fra X is the characteristic type of perseverative language described above for males with fra X.

Additionally, it has become a well-established fact that males with fra X are susceptible to anxiety disorders (Bregman et al., 1988, Levitas et al., 1983) and find eye contact aversive (Cohen et al., 1988, 1989; Bregman et al., 1988; Wolff et al., 1989). It is our belief, based on experimental results obtained by our group and others, that fra X is associated with avoidance of social interaction. This social avoidance is evident whether or not the individual is diagnosed as having autistic features (Cohen et al., 1988; Cohen et al., 1989; Cohen et al., 1991; Bregman et al., 1988; Einfeld et al., 1989; Wolff et al., 1989).

Males with fra X demonstrate this social avoidance through a conglomeration of associated behaviors such as gaze aversion, tactile defensiveness, overtly turning the body away during face to face social interaction and repetitive motor movements typically characterized by "anxious" rubbing of the body and rocking or hand flapping.

"Avoidance of social contact" appears to be a specific behavior that distinguishes fra X males with MR or Atypical Pervasive Developmental Disorder (PDD) from non-fra X males with similar developmental disabilities (Cohen et al., 1988). For example, fra X males with MR, PDD and autism were found to be more avoidant with strangers than were non-fra X autistic or PDD males. Fra X males also have been found to be more likely than non-fra X males to selectively avoid eye contact and not establish mutual gaze (Cohen et al., 1989). Wolff et al. (1989) reported that adolescent and young adult males with fra X turn their whole body away when shaking hands with others. Bregman et al. (1988) noted gaze aversion in 14 fra X males between the ages of 3-27 which they attributed to underlying anxiety rather than to autistic social dysfunction.

Thus, it seemed to us that we should begin to investigate the relationship between social avoidance and the production of perseverative language. This research is on-going in our laboratories. What is presented below are some preliminary results from a pilot study performed three years ago.

EYE CONTACT AND PERSEVERATIVE LANGUAGE

Sudhalter and Cohen (1989) investigated the relationship of eye gaze and the probability of production of deviant language among males with fra X. It was reasoned that heightened sensitivity to social gaze could cause fra X MR males to feel discomfort, which then could be manifested in an increased production of deviant repetitive language. Thus, some of the repetitive deviant language typically observed in this population could be an indication of underlying social anxiety rather than having its basis in deviant language processes.

For interactions with both familiar and unfamiliar adults, we found a greater probability that the fra X male would produce deviant repetitive language if the adult looked at the child while the child talked compared to when the adult turned away. Thus, we have some preliminary indication that there may be a relationship between sensitivity to eye gaze and the emergence of deviant repetitive language in the fra X male. However, deviant language was also observed when adults were not looking at the children, indicating that eye contact is not the only cause for the emergence of deviant language in males with fra X.

One major implication of this preliminary finding is that one *should not* force mutual eye contact between a male with fra X and another. Forcing the male to maintain eye contact (which is practically impossible) may promote the production of deviant language, a condition one would wish to avoid.

We now turn to the investigation of the various language competencies and their possible contribution to the production of perseverative language.

SYNTACTIC COMPETENCE

Although speech and language problems are apparent, syntactic skills are relatively unaffected in fra X. Paul et al. (1987), studying the language ability of males with fra X, autism and cultural/familial retardation, demonstrated that there was no difference in the syntactic ability or the knowledge of the sentence patterns of the language between these groups. Additionally, Scarborough et al. (1991) studied the syntactic ability of several groups of handicapped children, including children with DS, language impairment, autism and fra X. Two measures were used to assess the syntactic ability of the children, mean length of utterance (MLU) and the Index of Productive Syntax (IPSyn) (see Scarborough 1990 for a detailed description of the IPSyn). The ranges of IPSyn scores for the males with fra X were roughly comparable to the nonimpaired preschoolers. These data suggest that the fra X male presents with delayed but non-deviant syntactic skill. Sudhalter, Scarborough and Cohen (1991) also found that syntactic ability, as measured by IPSyn and MLU, was not correlated with the production of deviant language. Thus, we can conclude that inasmuch as the male with fra X *does not* seem to manifest difficulty with syntactic structures, an inability to complete a thought with the correct structure (e.g., noun versus verb, preposition versus adverb) *is not the prime cause for the production of perseverative language in males with fra X.*

Next to be presented are the studies that assessed receptive and expressive semantic competence in males with fra X and language age matched non-delayed children. This research was supported by a grant from the March of Dimes Foundation (12-259).

SEMANTIC COMPETENCE: (A) RECEPTIVE SEMANTIC COMPETENCE

Picture Categorization Task

Receptive semantic competence was assessed with a categorization task. Two groups participated in this study, males with fra X and non-delayed youngsters. The child was shown a group of eight pictures and asked to give the examiner all the examples of a particular category. There were two categories -- taxonomic and episodic. The taxonomic categories included fruits, toys, musical instruments, tools, clothing and animals. The episodic categories included such concepts as things mommy likes, things found in school, things found in a living room, things found at a birthday party, things taken on a picnic and things found in a kitchen. For instance, the pictures depicting the category "toys" showed a football, a basketball, a bicycle and a set of blocks. The pictures depicting the category things mommy likes showed clothes, pretty shoes, flowers and an obviously delicious meal.

Seven fra X and eleven non-delayed children were given the task. They were asked to identify the four pictures that belong in the category by giving them to the experimenter. Each category had 4 correct members and 4 distractors. A training set was presented. No one had any difficulty in performing the task.

There were two types of possible errors, inclusion and exclusion of examples. An inclusion error was identifying a distractor card as belonging to a category, and an exclusion error was not identifying one of the target cards. For the purpose of the discussion here, we will collapse across exclusion and inclusion errors. Results indicated there was no group difference in the number of errors committed ($p<.07$). The one significant finding was that more errors were committed when the children (fra X and non-delayed) were confronted with the episodic categories then when confronted by the taxonomic categories ($p<.02$). Thus we have some initial evidence to suggest that early category formation is approximately the same for males with fra X and language age matched non-delayed children.

SEMANTIC COMPETENCE: (B) RECEPTIVE SEMANTIC COMPETENCE

Sentence Verification

We wanted to explore how much the males with fra X knew about the concepts they had developed; therefore, we developed a sentence verification task that has the advantage of having low productive demands while assessing the semantic system of individuals. It assesses what the subject knows about the features of a semantic concept by requiring him to indicate simply if a sentence read to him is "true" or "false." For instance, some of the various features of the concept "tree" are that it is inanimate, grows, has branches, leaves or needles, cannot think or run and boys and girls can climb on it. The general purpose of the task was to ascertain if the child with fra X had acquired a complete semantic concept.

We also added eleven sentences with high associate compounds to gather some initial evidence as to non-delayed childrens' and fra X males' ability to block impulsive responding. Sentences with high associate compounds are such sentences as "Do birds see with their feathers?" and "Do elephants hear with their trunks?" in which a word (*bird*) is paired with another word (*feathers*) with which it is associated. In all the sentences which contained associate pairs, the correct answer was "no" or "false." We reasoned that if the child had difficulty blocking associative responding, then he should be "fooled" into answering "yes" or "true" to the sentences containing the high associate compound because the high associate would be primed and the child would have difficulty blocking the recognition of the word when heard.

Stimuli

A total of 139 sentences (72 yes/67 no) tested knowledge of animate (human and animal) and inanimate objects. The sentences assessed knowledge of super-ordinate category (e.g., "Is a cat an animal?"), physical ("Do birds have wings?") and cognitive attributes ("Can a cat read?").

Results

Four types of responses were possible within the sentence verification task: saying yes/true when the answer should be yes or true (**correct:CR**); saying no or false when the answer should be no or false (**correct rejection:RJ**); saying yes or true when the answer should be no or false (**false alarm:FA**) and saying no or false when the answer should be true or yes (**miss:MS**). Thus, two distinct types of errors are possible: false alarms and misses.

Sentences Without High Associate Compounds

Table 1 presents the percentages of each response type for each of the participants of this study, exclusive of the sentences with high associate compounds. The percentages for the response type and its error (e.g., correct and miss and correct rejection and false alarm) add to one hundred percent. Thus each line of the table adds to 200%.

Table 1
Results for the Sentences
Without the High Associate Compounds

Fragile X Males

	%CR	%FA	%RJ	%MS
1) fra X	87	19	81	13
2) fra X	96	7	93	4
3) fra X	88	25	75	12
4) fra X	95	29	71	5
5) fra X	90	22	78	10
6) fra X	97	29	71	3
7) fra X	85	27	73	15
8) fra X	98	6	94	2
9) fra X	100	6	94	0
10) fra X	93	5	95	7

Non-Fra X non-delayed 4-yr olds:

	%CR	%FA	%RJ	%MS
1) 4-yrs	86	2	98	14
2) 4 yrs	92	0	100	8
3) 4 yrs	97	10	90	3
4) 4 yrs	90	24	76	10
5) 4 yrs	91	24	76	9
6) 4 yrs	96	33	67	4
7) 4 yrs	94	5	95	6
8) 4 yrs	70	19	81	30
9) 4 yrs	97	12	88	3
10) 4 yrs	85	12	88	15
11) 4 yrs	96	13	87	4

As these results suggest, males with fra X appear to have similar knowledge concerning the concepts they have acquired as compared to non-delayed 4-year old children. This is indicated by the fact that there appears to be no difference in the percentage correct (fra X=93% vs 4-yr=90%) between the two groups. Additionally, there is no difference in the overall correct rejections (fra X=83% vs 4-yr=86%).

Sentences with High Associate Compounds

An analysis of the 11 sentences that contained the high associate compounds yielded significant differences between the fra X and non-delayed participants. Using a t test for related measures with the 11 sentences entered as the independent variable and the percentage of subjects from each group (fra X vs non-delayed children) who answered incorrectly as the dependent variable, it was found that significantly more of the subjects with fra X committed errors (saying "yes" when the answer was "no") than the non-delayed 4 year olds [$t(10)=5.6$, $p<.001$]. Table 2 presents the percentage of males with fra X and of non-delayed children who committed errors on each of the high associate sentences. Thus we have some initial evidence that males with fra X have difficulty blocking impulsive responding and this impulsive responding may be one of the causes of the production of perseverative language.

Table 2
Percentage of False Alarm Errors on Sentences With High Associate Compounds

	Fragile(X)	Non-delayed
Do people drink glass ?	.67	.09
Do babies wear bottles ?	.33	.09
Is a boy a girl ?	.33	0
Do birds see with their feathers ?	.78	.18
Can elephant see with their floppy ears?	.33	.09
Do elephants hear with their trunks ?	.44	.09
Do monkeys wear bananas ?	.11	.09
Do carrots like rabbits ?	.67	.64
Can papers write with pencils ?	.67	.27
Can webs spin spiders ?	.44	.18
Can a fire smell ?	.56	.27

Conclusions

We believe we have obtained two important preliminary findings: (1) no difference in the overall correct score and the correct rejection score between the two groups, and (2) significant differences on the sentences with high associate compounds. These results begin to delineate the nature of the semantic deficit in males with fra X, suggesting that males with fra X have at least as complete a semantic concept as do language age matched, non-delayed children and that males with fra X have difficulty inhibiting the activation of high associates, thus forcing miscomprehensions of sentences containing high associate compounds.

The next study presents the findings of productive semantic competence. Productive semantics is defined herein as the ability to choose the correct word from one's mental lexicon (internal vocabulary system) so as to produce a meaningful and well-formed thought. There are several underlying processes and competencies that compose semantic competence. Any one or combination of these processes (competencies) could be deficient in males with fra X, a malfunction, which, in turn, could lead to the production of deviant perseverative language. An example of such a deficit is observed when a longer period of time is needed to locate the "correct" word, resulting in "perseveration" on a known phrase or word as a form of place holder while searching for the more unfamiliar word. A second example would be an inability to locate the "correct" word (word recall or word retrieval deficits) in the mental lexicon. This deficit not only could result in "perseveration," but also in such behaviors as word substitution or in the production of phonemic place holders such as "Uh, uh, oh." Additionally, the individual could become more and more agitated (hyperaroused) when forced to engage within unfamiliar semantic domains. The resultant hyperarousal could cause the "perseveration," which then would be interpreted as a form of anxious behavior. This mechanism may be of particular relevance for topical perseveration where a person continues to discuss a particular topic (e.g., The Partridge Family, Bob Hope, The Rolling Stones) long after any new information had been added to the topic. Topical perseveration can be interpreted as a way of controlling the conversational context so as to converse within only familiar areas.

Obviously there are many consequences of deficits in productive semantics, and none are exclusive. However, the major point is that deficits in the processes composing productive semantics could begin to provide yet another part of the explanation for the deviant language production of males with fra X.

SEMANTIC COMPETENCE: B) PRODUCTIVE SEMANTIC COMPETENCE

Sentence Completion Task

We investigated the productive semantic ability of 11 males with fra X and 11 non delayed children (Sudhalter et al., 1992).

Materials and Methods

Subjects

Eleven males with fra X participated in the present study. The 11 males with fra X ranged in age from 6-41 years, and had an average Communication Age Equivalent (CAE) of 4 4/12 years as measured by the Vineland Adaptive Behavior Scale (Sparrow et al., 1984). Four of the males lived in residence homes and the remaining 7 lived at home with their parents and attended special education programs. The males with fra X were recruited from the patient population of the Institute for Basic Research in Developmental Disabilities.

Table 3

	DOB	CA	CAE	Residence
FX1	08/31/61	29;8	3;11	Group home
FX2	10/26/77	12;8	7;9	Parents
FX3	07/08/77	13;3	5;5	Parents
FX4	07/16/46	44;10	4;6	Group home
FX5	03/15/61	29;6	3;5	Group home
FX6	04/10/79	12;2	4;3	Parents
FX7	01/25/75	15;9	4;9	Parents
FX8	07/16/85	5;11	3;9	Parents
FX9	05/21/77	13;2	5;5	Parents
FX10	09/29/53	37;0	4;5	Group home
FX11	12/08/76	12;11	3;11	Parents

Table 3 provides the age at time of testing (CA), CAE and place of residence for the 11 fra X males who participated in this study.

In order for the males with fra X to participate in this study, they had to meet two major criteria. No one with a diagnosis of autistic disorder was included, and participants had to be able to engage in a three-four turn conversation (e.g., adding three or four pieces of information to a conversational topic).

For a comparison group we tested the youngest possible non-delayed children. Our pilot studies indicated that 4 year old children were the youngest non-handicapped age group who were able to perform the sentence completion task consistently. Therefore, eleven four-year old, non-delayed children (7 boys and 4 girls) from a day-care center on Staten Island participated in the study.

Stimuli

The stimuli consisted of 99 sentences divided among: (1) sentences that test factual knowledge and are contextually constrained (e.g.,"Meat is cut with ..."); (2) sentences that test sentient-experiential knowledge and are contextually constrained (e.g., "If you're hungry you..."); (3) factual sentences that are not contextually constrained (e.g, "People use hands to...") and (4) experiential sentences that are not contextually constrained (e.g., "Grown-ups think about...").

Training and Procedure

Ten of the eleven males with fra X were tested in a quiet, well lit room located at the Institute for Basic Research in Developmental Disabilities (NYS IBR/DD), and the eleventh male was tested in his home. The non-delayed children were tested at their day-care center, in a separate well-lit space the administration provided. All the participants were seated next to the investigator, with the entire test session videotaped. Subjects were asked to answer the following questions: "A mailman delivers ____________" and "Your name is __________." No subject had any difficulty completing these 2 sentences. Following this training, the 99 test sentences, ordered randomly, were presented. Testing took 30-45 minutes. All responses, regardless of quality, were positively reinforced.

Coding

There were five different codes. These codes were "correct" if the answer contained no error or only a preposition error in the answer; syntactic error if there was an error in sentence formation; semantic error if the answer contained: (c1) a purely associative filler (e.g., Birds see *with their feathers*), (c2) a category member substitution (e.g., Cats hear with their *nose*), (c3) a wrong word, (c4) a perseveration (e.g., responding with the same word or phrase regardless of sentence), or (c5) a change of topic. Responses could also be coded as (d) vague, too general or a paraphrase of the question (e.g, "Ice cream tastes *like ice cream*") and (e) no answer or, "I don't know."

Results

Five analyses of covariance, (covarying for CAE) were performed, with the number of the various error types (semantic, syntactic, vague and no response) as the dependent variables, group (non-delayed vs fra X) as the between subjects independent variable, and constraint (contextually constrained vs no contextual constraint) and knowledge type (factual vs sentient-experiential) as within subjects independent variables. There were no significant main effects for group for the syntactic, vague, no response or incomplete completion error types, indicating that the males with fra X and the non-delayed children were performing similarly with regard to these types of responses.

For semantic errors, two significant main effects were found. A group effect ($F(1/19)=41.20$, $p<.00001$) indicated that males with fra X committed *significantly more semantic errors* than the non-delayed four-year old children, and a constraint effect ($F(1/19)=12.65$, $p<.002$) indicated that more semantic errors were committed while trying to complete sentences with no contextual constraint than while trying to complete sentences that were contextually constrained. There was also an interaction between group and constraint ($F(1/19)=8.90$, $p<.008$), indicating that the relationship between semantic errors and sentence constraint was different depending on the group. Pooling across knowledge type, the mean number of semantic errors the males with fra X committed while trying to complete contextually constrained sentences was 1.91, compared to 4.5 for sentences that had no-contextual constraint (a difference in magnitude of almost three times). In contrast, the mean number of semantic errors the non-delayed children committed for the contextually constrained sentence was .50 and this error rate increased only to .73 when sentences had no-contextual constraint (a difference in magnitude of less than .5). Thus, fra X males were affected by the reduction in contextual constraint more than their non-delayed CAE matched peers.

Our non-significant group effects with regard to syntactic errors confirmed previous findings that fra X males do not manifest deviant competence in this area. Additionally, across the other non-semantic error types (vague, no response and incomplete completion) there were no differences found between the non-delayed males and the males with fra X. Thus, the creation of the sentence does not appear to cause the production of deviant perseverative language.

Our findings clearly suggest that the productive semantics ability of fra X males appears to be one specific area of deficit. Males with fra X produced significantly more semantic errors than did the non-delayed children. In other words, when the fra X male is confronted with the task of having to choose the correct word to complete a sentence, he will make more errors in choosing that word than language age matched, non-delayed children. Indeed, the fra X male will have less trouble if there are fewer options for correctly completing the sentence (e.g., "Meat is cut with a __________") than if the sentence is more open ended (e.g.,"Parents think about __________").

These results taken in conjunction with the results of the other studies suggest that (a) males with fra X have developed as complete a semantic knowledge as is needed to be able to complete sentences correctly yet (b) they have difficulty blocking high associates and (c) have difficulty in locating the correct word to complete a sentence (though this difficulty is lessened if the sentence is more constrained and less open ended). It is an important question as to why the fra X male is prone to producing semantic errors inasmuch as we have already determined that his semantic concept is at least as well formed as his non-delayed peer. One answer is that as a result of social anxiety or perhaps hyperarousal, the male with fra X is unable to block the activation of high associates (e.g., when someone says "up" "down" becomes somewhat activated in the mental lexicon). In non-delayed individuals this response to high associates is likely dampened so comprehension is not similarly hindered.

GENERAL CONCLUSIONS

What have we learned from the results of the studies cited above? For one thing we have learned that the major language deviance produced by males with fra X is perseverative language. Though to date we are not completely sure of what causes the emergence of perseverative language, we can definitely rule out deficits in syntactic competence as it was demonstrated in two research studies that males with fra X did not have any special difficulty with syntactic knowledge and that errors in syntax were not related to the emergence of perseverative language. Second, we can state with some certainty that semantic concept formation is also not an area of special deficit in males with fra X.

However, the two areas where linguistic deficits were evident were (1) in an inability to block the high associate and (2) not being able to successfully complete a sentence with the correct word. These two areas are probably related for in order to be able to correctly complete a sentence the child has to be able to attend to the complete sentence and thus be able to block high associate responding. Lastly, it was shown that the production of repetitive language in males with fra X was exacerbated by eye contact.

If we are to train and educate the fra X male we must be aware that he will respond with high associates and thus encourage him to listen to the whole sentence. It is imperative to make the learning environment as pleasant as possible for any child, and for the child with fra X this means a quiet, calm environment where people do not demand social interaction such as eye contact when the child is "overaroused." It is imperative to dearouse the aroused fra X male so he can listen and process what is being said to him and so that he will be successful in communicating to others.

It is also probable that the male with fra X may score artifactually low (or lower than his "true" ability) on "standardized" tasks which are socially demanding and require high productive competence. Professionals may be underestimating the verbal performance ability of these males. Lower expectations for males with fra X can and will have a detrimental effect on their overall achievement and on their overall life fulfillment. Therefore, we have to assist males with fra X to be able to demonstrate their true abilities.

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THE BEHAVIORAL PHENOTYPE OF FRAGILE X SYNDROME AND ITS ASSOCIATION WITH AUTISM

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> "When you hear hoofbeats, don't think of zebras!"
> ("... old medical school adage..."; Marmor, M.F. (1982).
> Wilson, strokes, and zebras, The New England Journal of Medicine, 528-535.)

> "Never assume that the sound of hoofbeats signifies a horse.
> Sometimes it may be a zebra"
> (Larsen, D. (Jan 14, 1986) describing issues in medical diagnosis.
> Los Angeles Times, Part 5, pg. 1, col. 1.)

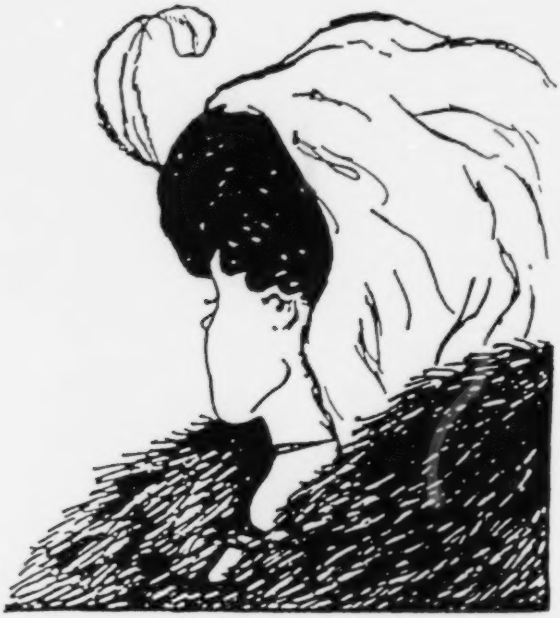

Figure 1. Is this a young woman or an old woman? Notice that this ambiguous figure can be interpreted as either depending on the way it is perceived by the viewer. This is directly analogous to the different ways in which the person with fragile X is perceived by different diagnosticians. After Leeper (1935).

As is obvious from the above quotes and from Figure 1, when two or more people hear and/or see the same event, they may not always agree on the meaning of what they perceived, particularly when the evidence is hard to objectively define and heavily influenced by personal experience. This is as true for witnesses at legal trials as it is for diagnosticians in medicine, psychology or psychiatry. This diagnostic dilemma is, in large part, at the heart of the controversy as to whether or not autism is associated with the fragile X syndrome (Blomquist et al., 1985, Bregman et al., 1988; Brown et al., 1982a; Brown et al, 1982b; Brown et al., 1986; Cohen et al., 1991; Einfeld et al., 1989; Fisch et al., 1986; Fisch, 1992; Gillberg, 1983; Goldfine et al., 1985; Levitas et al., 1983; Meryash et al., 1982; Partington, 1984; Payton et al., 1986; Piven et al., 1991; Venter et al., 1986; Watson et al., 1984; Wisniewski et al., 1985).

The purpose of this paper is to discuss, and attempt to answer (where possible), some basic questions:

1. What is autism?
2. Can anything be done to improve the reliability of the diagnosis of autism?
3. Is autism associated with fragile X syndrome? Why is this an important question?
4. What is happening at the genetic level which may be related to the behavior problems shown by people with fragile X syndrome?
5. What are the similarities and differences between persons with autism who do and don't have the fragile X syndrome?

WHAT IS AUTISM?

Autism was first recognized as a unique disorder by Leo Kanner (1943) in his seminal paper entitled *Autistic Disturbances of Affective Contact.* In that manuscript, Kanner clearly described 11 cases he had seen in his office that he felt described a unique entity. Through rich narratives of individual cases (filtered through Kanner's years of experience), the reader achieves some understanding of what it was that Kanner observed. I have abstracted some of these unique characteristics in Table 1. Basically, Kanner described children whom he felt had a primary disturbance in their ability to interact with significant others or to social situations "from the beginning of life." In addition, such children did not comprehend or use language in the same way as did non-handicapped or language-delayed children, i.e., for social communication. Instead, their language consisted of parroting back what was said to them with no evidence of comprehension, extreme literal interpretations of word meanings (e.g., "up" always meaning having oneself picked up), unusual ways of speaking (voice too soft or too loud or use of a questioning melody when a question wasn't asked) or constant talking about a single topic with insensitivity to the wishes of the conversational partner or amassing information about a single topic. Finally, bizarre reactions to the environment such as insistence that routines be maintained, food refusal, hypersensitivity to sounds or visual events or repetitive movements of the body were also described. It is important to emphasize that the behavior pattern that Kanner described was ***different*** from that shown by non-handicapped or developmentally delayed children.

Developmentally delayed children typically have problems with communication, but their comprehension and use of language is *delayed*, i.e., typical of a younger, non-handicapped child.

Table 1.
Kanner's Observations On Autism (Kanner, L. (1943). Autistic disturbances of affective contact. Nervous Child, 2:217-250.)

Sample: 8 boys; 3 girls

1. "inability to relate themselves in the ordinary way to people and situations from the beginning of life"
2. Of 8 speaking children, none used language to convey meaning
    a. echolalia and delayed echolalia
    b. affirmation by repetition
    c. literalness
    d. "personal pronouns are repeated as heard"
3. Excellent rote memories noted
4. "all powerful need for being left undisturbed" as exemplified by:
    a. food refusal
    b. loud noises and moving objects reacted to with horror
5. "anxiously obsessive desire for the maintenance of sameness" as exemplified by need for routines to be unchanged and/or furniture not rearranged
6. Monotonous and repetitive motions and verbal utterances
7. Good relation to objects - not to people
8. "intelligent physiognomies" (intelligent facial appearance)

Despite this early description of autism, it was not until 1980 that it was diagnostically recognized as its own entity in the Diagnostic and Statistical Manual of Mental Disorders (3rd Edition) or DSM-III (APA, 1980). Prior to 1980, autism was considered to be a form of childhood schizophrenia. Indeed, Fish (1977) considered it to be the earliest expression of schizophrenia. However, subsequent investigations documented very little similarity between autism and schizophrenia (DeMyer et al., 1981) and the condition was eventually recognized as a developmental disorder.

In the DSM-III (APA, 1980), there were three categories of autistic behavior under the umbrella term of "pervasive developmental disorder (PDD)": infantile autism, childhood onset pervasive developmental disorder and atypical developmental disorder.

Table 2

Diagnostic Criteria for Infantile Autism, Childhood Onset Pervasive Developmental Disorder and Atypical Pervasive Developmental Disorder according to DSM-III.

Infantile Autism

A. Onset before 30 months of age.
B. Pervasive lack of responsiveness to other people (autism).
C. Gross deficits in language development.
D. If speech is present, peculiar speech patterns such as immediate and delayed echolalia, metaphorical language, pronominal reversal.
E. Bizarre responses to various aspects of the environment, e.g., resistance to change, peculiar interest in or attachment to animate or inanimate objects.
F. Absence of delusions, hallucinations, loosening of associations, and incoherence as in Schizophrenia.

Childhood Onset Pervasive Developmental Disorder

A. Gross and sustained impairment in social relationships, e.g., lack of appropriate affective responsivity, inappropriate clinging, asociality, lack of empathy.
B. At least three of the following:
    (1) sudden excessive anxiety manifested by such symptoms as free-floating anxiety, catastrophic reactions to everyday occurrences, inability to be consoled when upset, unexplained panic attacks
    (2) constricted or inappropriate affect, including lack of appropriate fear reactions, unexplained rage reactions, and extreme mood lability
    (3) resistance to change in the environment (e.g., upset if dinner time is changed), or insistence on doing things in the same manner every time (e.g., putting on clothes always in the same order)
    (4) oddities of motor movement, such as peculiar posturing, peculiar hand or finger movements, or walking on tiptoe
    (5) abnormalities of speech, such as questionlike melody, monotonous voice

(6) hyper- or hypo-sensitivity to sensory stimuli, e.g., hyperacusis
(7) self-mutilation, e.g., biting or hitting self, head banging

C. Onset of the full syndrome after 30 months of age and before 12 years of age.

D. Absence of delusions, hallucination, incoherence, or marked loosening of associations.

Atypical Pervasive Developmental Disorder

This category should be used for children with distortions in the development of multiple basic psychological functions that are involved in the development of social skills and language and that cannot be classified as either Infantile Autism or Childhood Onset Pervasive Developmental Disorder.

As shown in Table 2, the descriptions of infantile autism and childhood onset pervasive developmental disorder are quite similar with the two differing primarily in age of onset. It should also be noted that some of the descriptors are "vague" and not as rich as those provided in Kanner's article. Atypical pervasive developmental disorder can be considered an "autistic-like" disability with no age of onset criterion.

As a result of problems with the use of DSM-III, the criteria for diagnosing autism were changed in 1987 in the DSM-III-R (APA, 1987) and these are shown in table 3. Primary changes are: 1) a reduction in the number of disabilities to two: autistic disorder and pervasive developmental disorder - not otherwise specified; 2) elimination of the term "infantile"; 3) increased specificity of diagnostic criteria both in terms of minimal number of symptoms and their descriptions; 4) elimination of 30 months of age as a cut-off and, instead, emphasis on onset in infancy or early childhood; and 5) elimination of schizophrenia as an exclusionary criterion, i.e., it was recognized one can have both autism and schizophrenia.

Table 3

Diagnostic Criteria for Autistic Disorder and Pervasive Developmental Disorder Not Otherwise Specified according to DSM-III-R. For DSM-III-R Autistic Disorder, at least eight of the following sixteen items are present, these to include at least two items from A, one from B, and one from C. For Pervasive Developmental Disorder Not Otherwise Specified, there is a qualitative impairment in the development of reciprocal social interaction and of verbal and nonverbal communication skills, but the criteria are not met for Autistic Disorder, Schizophrenia, or Schizotypal or Schizoid Personality Disorder.

Note: Consider a criterion to be met *only* if the behavior is abnormal for the person's developmental level.

A. Qualitative impairment in reciprocal social interaction as manifested by the following:
   (1) marked lack of awareness of the existence or feelings of others (e.g., treats a person as if he or she were a piece of furniture; does not notice another person's distress; apparently has no concept of the need of others for privacy)
   (2) no or abnormal seeking of comfort at times of distress (e.g., does not come for comfort even when ill, hurt, or tired; seeks comfort in a stereotyped way, e.g., says "cheese, cheese, cheese" whenever hurt)
   (3) no or impaired imitation (e.g., does not wave bye-bye; does not copy mother's domestic activities; mechanical imitation of others' actions out of context)
   (4) no or abnormal social play (e.g., does not actively participate in simple games; prefers solitary play activities; involves other children in play only as "mechanical aids")
   (5) gross impairment in ability to make peer friendships (e.g., no interest in making peer friendships; despite interest in making friends, demonstrates lack of understanding of conventions of social interaction, for example, reads phone book to uninterested peer)

B. Qualitative impairment in verbal and nonverbal communication, and in imaginative activity, as manifested by the following:
   (1) no mode of communication, such as communicative babbling, facial expression, gesture, mime, or spoken language
   (2) markedly abnormal nonverbal communication, as in the use of eye-to-eye gaze, facial expression, body posture, or gestures to initiate or modulate social

interaction (e.g., does not anticipate being held, stiffens when held, does not look at the person or smile when making a social approach, does not greet parents or visitors, has a fixed stare in social situations)

(3) absence of imaginative activity, such as playacting of adult roles, fantasy characters, or animals; lack of interest in stories about imaginary events

(4) marked abnormalities in the production of speech, including volume, pitch, stress, rate, rhythm, and intonation (e.g., monotonous tone, questionlike melody, or high pitch)

(5) marked abnormalities in the form or content of speech, including stereotyped and repetitive use of speech (e.g., immediate echolalia or mechanical repetition of television commercial); use of "you" when "I" is meant (e.g., using "You want cookie?" to mean "I want a cookie"); idiosyncratic use of words or phrases (e.g., "Go on green riding" to mean "I want to go on the swing"); or frequent irrelevant remarks (e.g., starts talking about train schedules during a conversation about sports)

(6) marked impairment in the ability to initiate or sustain a conversation with others, despite adequate speech (e.g., indulging in lengthy monologues on one subject regardless of interjections from others)

C. Markedly restricted repertoire of activities and interests, as manifested by the following:

(1) stereotyped body movements, e.g., hand-flicking or -twisting, spinning, head-banging, complex whole-body movements

(2) persistent preoccupation with parts of objects (e.g., sniffing or smelling objects, repetitive feeling of texture of materials, spinning wheels of toy cars) or attachment to unusual objects (e.g., insists on carrying around a piece of string)

(3) marked distress over changes in trivial aspects of the environment, e.g., when a vase is moved from usual position

(4) unreasonable insistence on following routines in precise detail, e.g., insisting that exactly the same route always be followed when shopping

(5) markedly restricted range of interests and a preoccupation with one narrow interest, e.g., interested only in lining up objects, in amassing facts about meteorology, or in pretending to be a fantasy character

D. Onset during infancy or childhood

These changes have made autism easier to define, especially in adults. However, it may be that autism as defined by the DSM-III-R is not the same as autism defined by the DSM-III (Volkmar et al., 1988). The constant changes in criteria through the years also suggests that researchers may not always have been studying the same classes of children, even though their labels were the same. Thus, what we think we know about "autism" may only be true of subsets of children or subsets of behaviors that consistently appear in the various diagnostic criteria.

It should also be evident, at this point, that the disorder called autism is defined on the basis of a *pattern of behaviors* and *not* on the basis of "organic" criteria such as disorders in one's chromosomes, hormones, electrical activity of the brain, etc. In other words, autism, at this stage of sophistication, is a "categorical" diagnosis that says nothing about the "real reason" or "etiology" for this behavioral condition. However, autism has been associated with a variety of organic conditions including phenylketonuria (Friedman, 1969), maternal rubella (Chess, 1971), herpes encephalopathy (Gillberg, 1991), neurofibromatosis (Gillberg & Forsell, 1984) and tuberous sclerosis (Reiss et al., 1986), among others (Coleman, 1976).

CAN ANYTHING BE DONE TO IMPROVE THE RELIABILITY OF THE DIAGNOSIS OF AUTISM?

At this point, it is reasonable to ask some obvious questions:
"How do we know there is such a thing as autism and why is it so difficult to define?"
"If the label says nothing about what causes the condition, why keep the diagnosis?"
"Can anything be done to improve the reliability of diagnosis?

In point of fact, autism, in *most* cases, is only difficult to define when one is forced to rely on set diagnostic criteria and behavior checklists. Experienced diagnosticians quickly recognize and agree on the condition when they see it and hear it (Campbell, personal communication; Rapin, 1987). According to Rapin (1987), what these diagnosticians seem to be responding to are the "core" characteristics of the disorder, i.e., features that form unique patterns once a person has had enough experience with the variety of ways in which the disorder can present itself. Providing verbal descriptions of these core features is the difficult process.

In a direct analogy shown in Figure 2, imagine trying to verbally describe to a person who has never seen English printing or script why the upper-case printed R on the top row is the same as the first and last letters on the second row (lower-case R and upper case script R) but *not* the same as the middle letter on the second row (upper case P). Not easy, is it? Clearly, the "P" is more physically similar to the printed, upper-case "R" than is the printed, lower-case "r" and therefore physical similarity, alone, is not enough. It is much easier to give a novice many different visual examples, in a variety of contexts, until he or she can recognize the letter. In fact, after some experience, people can recognize letters that are partially obliterated on the basis of context. For example, people recognize that the word "pan" is actually a misspelled "ran" in the sentence, "The boy pan down the street." Thus, in this case, a "p" is not read as a "p" on the basis of its physical features but as an "r" based on context. The "core features" that people recognize as "R-ness," in the face of such variety and noise, cannot easily be put into words and this is also the crux of the problem with the diagnosis of autism.

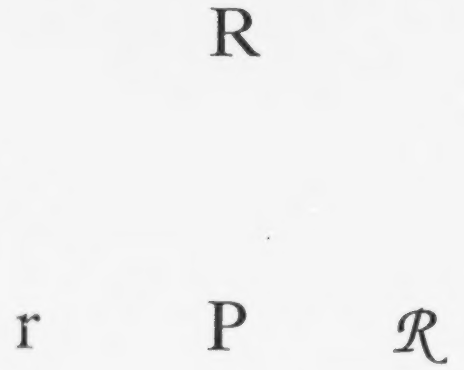

Figure 2: Why is the capital, printed letter "R" the same as the letters on the left and right in the row but not the same as the middle letter "P"? See text.

In a somewhat similar fashion, diagnosticians learn to recognize autism by being shown examples of the condition by their mentors. They learn autism as it is expressed in a young boy or girl with an IQ of 50, a 20 year old man with IQ of 20 or a 25 year old man with an IQ of 115. As they experience more and more examples, their skills improve and they learn to recognize "autism." But they have difficulty putting into precise, quantitative or even qualitative fashion what it is they are seeing so that it can be communicated to others who have never experienced the condition or its variety of expression. In fact, even with explicit written criteria and experience, the decision to classify an individual as autistic is influenced by one's personal beliefs. For example, many severely impaired, developmentally disabled individuals display behaviors which might qualify them as autistic to some diagnosticians. To others, the behaviors are categorized as "autistic features" and attributed to the person's retardation, a phenomenon that may be called "diagnostic overshadowing" (Reiss et al., 1982) if the diagnosis of autism is, in fact, appropriate. Interestingly, it is rarely the case that a person's severe retardation is attributed to his/her "severe" autism.

Parents, as well as experienced diagnosticians, also recognize autism, in many cases. This is particularly the case when there are multiple occurrences within families, an event that is not unusual (Jones & Szatmari, 1988; Ritvo et al., 1989a and 1989b; Smalley, 1991). Such children are clearly different and present with such unusual behaviors and lack of interest in social interactions that they often take a severe toll on the emotions of their parents, siblings and extended family. Parents are often bewildered and feel guilty that they may have inadvertently done something wrong to "cause" their child's condition. Studies have indicated that nothing could be further from the truth (DeMyer, 1981). Indeed, in my experience, the failure of parents or professionals to comprehend what caused this disturbance is more troubling than the diagnosis itself.

Further, recent research suggests that many children with autism can be helped with intensive, behavior modification programs (Lovaas, 1987). Thus, for practical reasons, diagnosis is important for families.

For researchers, diagnosis is also important. The first step in science is to group like things together in the hope of arriving at some sort of order from a bewildering array. The fact that one can recognize an individual with autism no matter what the age, sex, race or intellectual level of the person suggests that some *common core features* are recognizable. If we can better define these core features, we are likely to be in a better position to relate autism to organic, neuropsychological and, perhaps, computational neuropsychological (i.e., neural network simulations of information processing style - see below) models of the condition. It is also possible that the more we understand autism, the more we will also understand normal child development.

As we have noted elsewhere (Cohen et al. submitted), while experienced clinicians may intuitively recognize the "core behavioral syndrome of autism" (Rapin, 1987), they do not yet know or agree on the definition of the features yet alone on how best to measure them with some emphasizing social deficits (Wing & Atwood, 1987), others emphasizing language (Rutter et al., 1971) and still others emphasizing a disturbance in sensory and/or perceptual systems (Ornitz & Ritvo, 1968). Further complicating the matter is the fact that our measures of behaviors associated with autism are inherently "noisy", i.e., two different raters seeing the same person and using the same rating instrument may often yield very different scores. Finally, the diagnostic systems (such as the DSM-III-R) that are used to arrive at a diagnosis assume that this very complex decision can be made by adding a group of observations together to come up with a cut-off score. However, as I have indicated above, recognizing **any** pattern, be it the letter R or a pattern of behaviors, is a complex process. How likely is it that one could describe how to recognize the letter R, in a variety of contexts, by adding up its features?

Recently, a new methodology has been developed which may help to solve these problems. Computer programs have been developed that attempt to mimic, at a very primitive level, the function of the nervous system and are known as "neural networks" (Rumelhart & McClelland, 1986). These neural networks have interesting properties. First, like a diagnostician, they are "taught" to discriminate patterns by repeated exposure to correct and incorrect features until they form their own rules which may then lead to the correct results. The rules they infer are decipherable and therefore could possibly help define the core deficits of autism. Second, information is stored (memory) in such programs in a "distributed" fashion throughout the network. As a result, the network tends to be immune to noisy or missing input. Finally, information is processed in a complex, non-linear fashion by the network and, therefore, more closely parallels the pattern recognizing mechanisms of living creatures. Others have found such networks to be much better categorizers than more traditional statistical techniques and to be useful in complex medical issues such as predicting survival time after coronary artery bypass surgery (heart surgery) (Goodman et al., 1992).

We (Cohen et al., submitted) have recently explored the utility of neural networks to accurately discriminate persons with autism from a matched group (matched on overall age and level of motor skills development) of persons with mental retardation without autism based on information supplied by parents in a structured interview. The interview was scored and based on an instrument we recently constructed, the Autistic Behavior Interview. The network results were compared with more traditional statistical classification techniques (discriminant function analysis). Our results indicated that the network was significantly superior to the discriminant analysis procedure not only in learning to discriminate cases correctly but also in accurately predicting cases that were not part of training. In these studies, the network model accurately predicted 93% of all new cases whereas the discriminant analysis procedures accurately predicted only 81% of all new cases.

Of somewhat greater interest was the observation that in order to arrive at this level of accuracy, the network we used learned that it had to sub-divide the autistic group into three different patterns of behavior. These behavior patterns, in turn, reflected increasing levels of intellectual functioning in our autistic sample and were similar in form to behavior sub-types described earlier by the well-known British psychiatrist, Lorna Wing (Wing & Atwood, 1987).

These results are encouraging. They suggest that it is possible to reliably diagnose autism *and* to describe the "core" behavior patterns that comprise the overall diagnosis. Perhaps these neural networks will help solve the fragile X-autism dilemma.

IS AUTISM ASSOCIATED WITH FRAGILE X SYNDROME? WHY IS THIS AN IMPORTANT QUESTION?

In most cases, the cause or causes of autism remain unknown. Identical twin studies (Folstein & Rutter, 1977; Ritvo et al., 1985) suggest a strong genetic influence but it has been difficult to isolate the gene or genes responsible. As indicated above, autism has been associated with a number of different organic etiologies, both genetic and viral, but these usually represent a small percentage of the total and these causes are usually not specific to autism. That is, disorders such as fragile X, maternal rubella and phenylketonuria are associated with autism, but they are also associated with other handicaps including mental retardation. This raises an important question:

> Do organic disorders such as fragile X syndrome cause autism or do they cause "autistic features" often associated with mental retardation (Einfeld et al, 1989; Einfeld & Hall, 1992)?

It is known, for example, that severe impairment in social interaction is more prevalent among children with severe mental retardation (Wing & Gould, 1979). Clearly, if these organic disorders merely cause "non-specific" brain damage with severe impairment in behavior and cognitive functioning as a consequence, the observation of an association with autistic features would be uninteresting. What, in fact, is the nature of the association of autism with mental retardation?

When one starts with a sample of persons with autism, most (about 70 to 80%) children and adults will be found to function in the "retarded" range, i.e., an IQ below 70 and levels of adaptive functioning (self-care, job skills, etc.) that are below normal. In an often cited study, Wing and Gould (1979) also examined the opposite question. What is the prevalence of autism among retarded children? Wing and Gould reported that the prevalence of autism varied with the range of *non-verbal* cognitive impairment as follows: profound (IQ<20): 4.5%; severe (IQ 20-34): 14.7%; moderate (IQ 35-49): 14.3%; mild (IQ 50-69): 0.4%; and non-retarded (IQ≥70): .003%.

Clearly, autism is associated with mental retardation, but the association is non-linear with smaller percentages at the lower and upper limits of the IQ distribution. Moreover, Wing and Gould's results suggest that autism, per se, is a better predictor of retardation than retardation is of autism since the percentage of autistic children who are functioning in the retarded range is greater than the percentage of children with retardation who are autistic. Therefore, if anything, some children may be severely retarded "because" they are autistic, rather than the other way around. This makes sense when one realizes that autism is characterized by qualitative impairments in social interaction and language, the two major ways in which children learn about the world around them. Actually, however, both autism and mental retardation are *names* for behavior patterns, *not* etiologies or explanations as others have suggested (Einfeld et al., 1992).

Wing and Gould also reported that social impairment, by contrast, was most impaired in the lowest functioning groups. In fact, 96% of profoundly retarded persons were socially impaired. Therefore, Wing and Gould distinguished social impairment, *per se*, from autism. Further, Wing and Gould did note that severely and profoundly retarded children can be found who were not considered socially impaired because their social skills matched their overall mental age, i.e., their skills were what one would expect of an infant or young child and were therefore not deviant.

What about the association of autism with fragile X syndrome? What percentage of children with fragile X have autism and what percentage of children with autism have fragile X syndrome? These questions have been examined by a number of authors and our group has

Overall, we recently reported that when large enough sample sizes are examined, about 8% of unrelated males with autism test positive (cytogenetically) for fragile X and about 15 to 16% of males with fragile X syndrome have autism (Cohen et al., 1991a; Hagerman et al., 1986). Interestingly, we also reported that about 9% of females with autism also test cytogenetically positive for fragile X syndrome (Cohen et al., 1989a).

It should be noted that the percentage of males with fragile X that we, and others, have found to be autistic is about the same percentage of persons with retardation that were autistic in Wing and Gould's (1979) study. Similar observations have been reported by Einfeld et al. (1989). Does this mean that autism and fragile X syndrome are *not* associated, as Einfeld et al. (1989) and Einfeld and Hall (1992) have suggested? Actually, it means that knowledge that a male has fragile X is about as informative in predicting autism as is knowledge that a male has retardation, i.e., *that fragile X is not specific to autism and mental retardation is not specific to autism*. It does *not* imply that fragile X and autism are not associated any more than it implies that autism and mental retardation are not associated, as we have argued elsewhere (Cohen et al. 1991a). The fact that two percentages are similar implies nothing about the *association* of the entities that make up the percentages although it does indicate the relative strength of the association. Actually, the figure of 15% of fragile X males with autism is about 300 times the overall population rate of autism which is, in North America and Europe, about 4 to 6 in every 10,000 cases (Zahner et al., 1987). More recent arguments of a higher upper limit for autism (Fisch, 1992) rely on population prevalence statistics from Japan. Reports on the prevalence of autism in Japan are, for as yet unexplained reasons, unusually high.

When I examined 20 published estimates of the prevalence of autism across countries, I found a significant effect related to geographic region (F(2,18)=5.26, p=.008). Specifically, almost all of the prevalence figures for autism from Japan (Hoshino et al., 1982; Ishii & Takahashi, 1983; Matsuishi et al., 1987; Sugiyama & Abe, 1989; Tanoue et al., 1988) (N=5; mean rate=14.05/10,000) were significantly higher than those studies from Europe (Bohman et al., 1983; Brask, 1970 cited in Ritvo et al., 1989a; Cialdella & Mamelle, 1989; Gillberg, 1984; Gillberg, 1991; Lotter, 1966; McCarthy et al., 1984; Steinhausen et al., 1986; Steffenberg & Gilberg, 1986; Wing et al., 1976; Wing & Gould, 1979) (N=11; mean rate=5.51/10,000 - Unequal N LSD test p=.01) or North America (Bryson et al., 1988; Burd et al., 1987; Ritvo et al., 1989a and 1989b; Treffert, 1970) (N=4; mean rate=4.59/10,000 - Unequal N test p=.011), with the latter two not differing significantly from each other (Unequal N LSD test p=.94), as shown in Figure 3 This apparent effect should be investigated further given the unique history of Japan. Whether these differences are due to genetic, environmental or both factors or to methods of diagnosis, however, remains to be determined.

Based on the above arguments, autism is associated with the fragile X syndrome but it is not the only, or indeed, the major associate of this etiology. Why, then, is the association important?

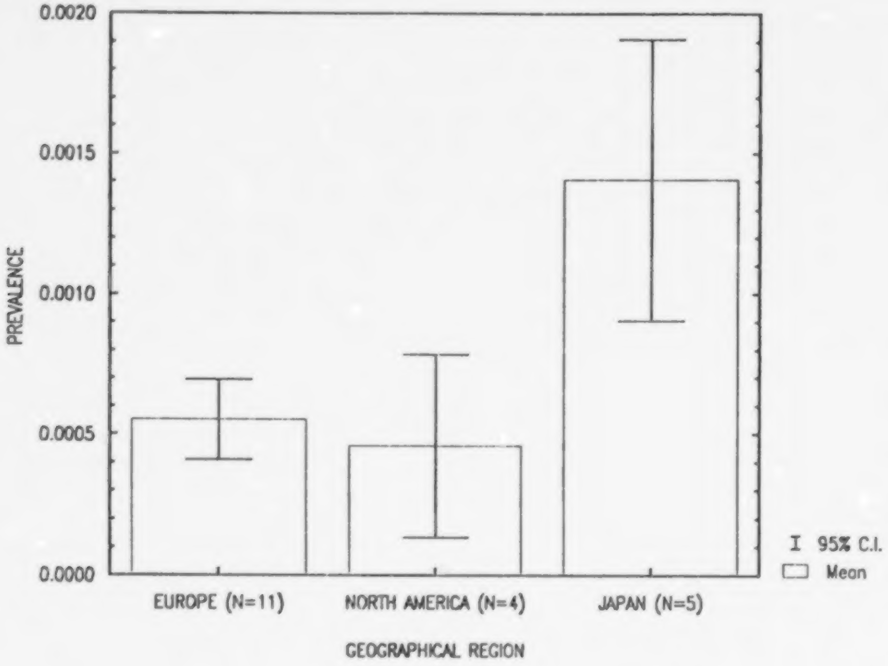

Figure 3: Mean prevalence of autism and the 95% confidence intervals across studies for different geographical regions of the world based on published data. N indicates the number of different studies that make up each bar in the graph.

As noted above, autism is a puzzle, not only in terms of the bizarreness of the behavior and its traumatic impact on families but also in terms of its cause or causes. Correlation, as we all know, is not the same as causation. *Simply because autism is associated with the fragile X syndrome does not imply that fragile X causes autism in a direct manner.* It may be that persons who have fragile X syndrome are, in turn, more susceptible to other etiologies which cause their autism. We have no evidence for this hypothesis at present. However, it is precisely because the fragile X syndrome does not always result in autism that it provides a unique opportunity to ask important questions about cause and effect such as, "In families with fragile X, where environmental differences across relatives are minimized, why is it that some persons with fragile X develop autism whereas their equally cognitively impaired fragile X cousins or siblings do not?" Recent research into the nature of the fragile X mutation may, eventually, provide an answer.

WHAT IS HAPPENING AT THE GENETIC LEVEL WHICH MAY BE RELATED TO THE BEHAVIOR PROBLEMS SHOWN BY PEOPLE WITH FRAGILE X SYNDROME?

Investigators have recently identified the region of the X chromosome which is affected, the nature of the mutation, i.e., its abnormal methylation pattern and instability (Oberle et al., 1991; Yu et al., 1991), the CGG repeat sequence and the gene, FMR-1 (Verkerk et al., 1991), which appears to be "turned off" and, therefore, presumably causes the syndrome by its lack of expression. The function of this gene, at the time this manuscript is being written, has not yet been elucidated. It appears that, however, this FMR-1 gene is not turned off in all of the cells in some affected individuals. Some cells seem to have a functioning gene. Such individuals who have both inactive and active genes are referred to as being "mosaic" for the fragile X syndrome. Of affected individuals, such mosaic cases represent less than 20% of the total (Rousseau et al., 1991) and have been thought to represent a "higher functioning" sub-type of the syndrome. Until recently, however, autism has not been examined in such individuals.

At the Third International Fragile X Conference, we (Cohen et al., 1992) reported in a poster that fragile X affected males with autism were mosaic (based on examination of white blood cells) for the fragile X mutation much more frequently (6 out 10 cases) than a randomly selected fragile X male control group (5 out of 22 cases; Fisher's Exact p=.05). We also noted that fragile X affected males who have members of the family who are both autistic and fragile X also were more likely to be mosaic. When these persons were combined with the cases with both fragile X and autism, 14 out of 22 were mosaic which was significantly different from the random control group ($X^2(1)=7.5$, p=.006; Fisher's Exact p=.007).

This very preliminary observation suggests that mosaicism may cause a *variable* outcome - "high functioning" persons in some families and autism in others. We did observe that mosaic persons did have higher levels of language functioning but this was true only in those under 17 years of age. These observations also imply that mosaicism *may* be a familial trait. Our sample size was small, however, and therefore these observations clearly require replication. It may also be that autism is related not just to mosaicism but also to the relative amount of active to inactive cells and/or the size of the CGG repeat sequence (C. Laird, personal communication). It is also the case that blood samples may not reflect what is happening in other tissues such as the central nervous system and this may explain why mosaicism was not specific to the family members with autism.

Thus, there is some evidence that we *may* be able to relate the genotype of fragile X to autism. If so, this could begin to help unravel the sequence of events from genes to brain structure and function to behavior (Hinton et al., 1991; Reiss, 1988; Smalley, 1991). Once more is known, we may have, at some point, a truly rational biologic treatment for one sub-type of autism, just as we do for phenylketonuria. Further, if fragile X autism is similar to other causes of the disorder, it is hoped that it may provide a useful model for understanding the biological mechanism or mechanisms for this bizarre condition.

But is the type of autism that is associated with the fragile X syndrome the same, behaviorally, as the type of autism associated with other organic conditions?

WHAT ARE THE SIMILARITIES AND DIFFERENCES BETWEEN PERSONS WITH AUTISM WHO DO AND DON'T HAVE THE FRAGILE X SYNDROME?

I have argued above that there is an entity called autism which can be identified and that is common across all levels of ability. This would suggest that there may be common brain regions (final common pathways) which are damaged by a variety of different etiologies, including fragile X (Cohen et al., 1991a; Smalley, 1991; Wing & Gould, 1979). However, in some individuals with fragile X syndrome, the mutation appears to cause differences in other areas of social functioning and these differences have relevance for treatment.

In a series of studies, we (Cohen et al., 1988; Cohen et al., 1989a; Cohen et al., 1991a, 1991b and 1991c) and other investigators (Bregman et al., 1988; Einfeld et al., 1989; Wolff et al., 1989) have noted that the fragile X syndrome is strongly associated with avoidance of social interaction. Behaviorally, social avoidance in the male with fragile X syndrome presents as specific avoidance of eye contact, tactile defensiveness and actively turning the body away or escaping during face to face social interaction. This behavior is evident even in males with fragile X who are not autistic and is particularly evident when interaction occurs with unfamiliar persons (Cohen et al., 1988).

It is our impression that, unlike persons with autism who do not have fragile X, males with fragile X syndrome avoid social interaction because they seem uncomfortable or anxious about the social requirements of making eye contact or being touched, i.e., they seem overly aroused by the stimuli involved in social interaction in a manner which resembles that described in premature infants (Field, 1979). Dr. Vicki Sudhalter, psycholinguist as well as friend and colleague, feels, in addition, that some of the tendency for males with fragile X to engage in perseverative speech may also be reflective of this social anxiety (these proceedings).

Non-fragile X persons with autism, by contrast, appear, in most cases, to be oblivious or insensitive to the requirements of social interaction. They become agitated during social interaction, it seems, either because they are being imposed upon and want to do something else or because others do not understand their needs, i.e., they appear frustrated for different reasons than the male with fragile X.

If true, these observations suggest that fragile X *may* cause autism by a different pathogenetic mechanism than other etiologies (Cohen et al., 1991a). Alternatively, these social avoidance problems may be an *additional* problem which needs to be taken into account in therapy for males with fragile X syndrome. They also suggest that medications which reduce arousal level may be helpful, in some cases, as a treatment for hyperarousal. Indeed, we have found that beta blockers such as propranolol, a drug used to treat high blood pressure, can be helpful (when there are no medical or psychiatric contraindications) in treating rage outbursts in males with fragile X when used in combination with small doses of haloperidol (Cohen et al., 1992).

SUMMARY

Since its earliest description, autism has been a diagnostic dilemma. I have tried to highlight here some of the problems inherent in differential diagnosis and have proposed a tentative solution through the use of neural networks. It is hoped that this methodology will enable researchers to use a common, standardized technique to arrive at a diagnosis and to understand the unique patterns of behavior which may be evident in behavioral sub-types of autistic disorder.

There is increasing evidence for the role of organic factors in the etiology of this severe disabling condition which affects so many children and which can have severe impacts on the family. When we understand the mechanism or mechanisms from an organic point of view, a rational biologic treatment may, theoretically, be developed. Since the mechanism by which organic factors cause autism is unknown, however, treatment remains symptomatic and empirical.

The most interesting relation, thus far, in terms of understanding cause-effect mechanisms, is that between autism and the fragile X syndrome. There are differences between persons with autism who have fragile X and those where etiology is unknown. This may be important in terms of treatment and in terms of understanding the mechanisms responsible for autism in those afflicted with the fragile X syndrome. However, behaviorally, there are also global similarities between these two groups. Because of this fact and because of the unique inheritance patterns associated with the fragile X syndrome, all children with autism of unknown etiology should be tested for fragile X. Newer methods of detection should dramatically reduce the cost (these proceedings).

More studies are needed which examine the behavioral sequelae of well-known organic conditions such as the fragile X syndrome or Down syndrome. Studies which only examine groups with unknown etiology will not help us to understand cause and effect. *Mental retardation is an effect, not a cause and therefore does not serve as an explanation for another behavior problem.* As we learn more about the FMR-1 gene and its consequences for the structure, function and computational ability of neuronal systems, it may be possible to more fully understand how and why autism appears in some individuals but not in others and, eventually, to provide a rational, as opposed to empirical, treatment for this disorder. Perhaps understanding the mechanisms involved in the fragile X-autism connection will also provide a useful model to help us understand links between autism and other organic etiologies, be they genetic, viral or other insults to the central nervous system.

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SOCIAL-EMOTIONAL CHARACTERISTICS OF ADULT MENTALLY RETARDED MEN WITH FRAGILE X SYNDROME

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INTRODUCTION

Since the delineation of the fragile X syndrome, a great deal of attention has been paid to its association with social-emotional features and behavioral difficulties. The social functioning of fra X males has been extensively described and characterized by autistic behavior, attention problems, hyperactivity, impulsiveness, anxiety and self-injuring behavior (Bregman et al., 1987; Bregman et al., 1988; Dykens et al., 1989; Hagerman et al., 1986; Hagerman, 1987; Levitas et al., 1983; Reiss & Freund, 1990). Temperament, on the contrary, is a highly neglected dimension in the study of the fra X personality, which has never been investigated comprehensively. In clinical reports, fra X males were mostly typified as friendly, cheerful and pleasant, although emotional outbursts, stubbornness and mood lability occur.

Controlled studies of social-emotional characteristics of adult fra X males, in which they are compared with a group of fra X negative persons with a non-specific type of mental retardation, are rare (Dykens et al., 1988; Gielen et al., 1988). Other comparative studies include younger fra X boys (Borghgraef et al., 1987, 1990; Einfeld et al., 1989) or are limited to a detailed exploration of some very specific behavioral features such as mutual gaze aversion and repetitive motor stereotypes (Cohen et al., 1988, 1989, 1991) or an unusual non-verbal greeting behavior (Wolff et al., 1989).

This report addresses some results of a study concerning social-emotional functioning of mentally retarded fra X adults. The following questions were in the focus of attention. Firstly, we intended to describe the profile of positive and/or negative characteristics of temperament and character which is consistent for and can be confirmed in a large group of fra X adults. Is this behavioral pattern specific for the fra X syndrome or merely the consequence of a general developmental delay? In other words, we wanted to point out specific social-emotional aspects to differentiate fra X males from other subgroups of mentally retarded persons. Finally, we have tested whether certain characteristics are more prominent in some age groups or with some levels of mental retardation.

SUBJECTS

Two groups of subjects participated in this study. Fifty-eight fra X men were all detected in systematic genetic diagnostic surveys performed by the Centre for Human Genetics (Leuven, Belgium) in 13 different residential institutions and day care centers for mentally retarded persons (Dereymaeker et al., 1988; Fryns et al., 1984, 1986, 1990). All of them had a cytogenetically (using M 199 lymphocyte cultures and screening 100 cells for the presence of an Xq27.3 fragility) confirmed diagnosis of fra X syndrome (mean fragility 12.27%, SD 9.87). The control group of the same size mainly consisted of cytogenetically fra X negative mentally retarded men with an unknown etiology of mental retardation (60.3%) and of men whose mental handicap was caused by a non-genetic factor, mostly pre-, peri- or postnatal factors and infections (39.7%).

Fra X and control subjects were all men, matched for chronologic age, period of institutionalization, actual living situation and level of cognitive function. Ages ranged between 21 and 67 years, with an average of approximately 40-41 years. They were always selected from the same institution and predominantly from the same groups. The period of institutionalization was similar in both groups and averaged 22.5 to 26 years (range 0-60 years). The mean mental age on the McCarthy or the Bayley Scales of Mental Abilities (Van der Meulen & Smrkovsky, 1983; 1985) was 3 years 4 to 5 months (range 8 months - 7 years). Approximately 2/3 of the groups were severely mentally retarded (MA 2-5 years), the others were to be found in the profound (≤ 2 years) or moderate (5-8 years) range.

METHODS

Staff members at the institutions who were familiar with the subjects filled in 4 behavioral scales. The Dutch TVZ-scale (Blok et al., 1990) of temperament for mentally retarded was used to examine a spectrum of personal characteristics that typifies the way in which someone behaves. Items are grouped in 7 dimensions of temperament. The Dutch SGZ-scale (Kraijer & Kema, 1981) reviews the frequency of physical aggressive, verbal and mixed forms of maladaptive behavior. Observations of autistic-like features were made by the Autism Behavior Checklist (Krug et al., 1980), a questionnaire for determining the level and the nature of behavioral features that are frequently observed in autistic populations, ordered in 5 subdomains. Finally, with the second part of the AAMD Adaptive Behavior Scale (Nihira et al., 1975) the level of socially undesirable behavior was evaluated in 13 different domains.

Results in raw scores or standard scores were statistically analyzed by SAS-computer program (1982), using student's t-tests for comparing the fra X and the control group, Student's t-tests for pairwise differences between dimensions or subscales in both group, chi^2-tests for association of fra X or control status with categorical variables and ANOVA F-tests for effects in a 2 x status and 3 x age or in a 2 x status and 4 x cognitive level design with HSD q-tests for Tukey a posteriori pairwise comparisons. Age groups are 21-35 (n = 25/26), 35-50 (n = 15/16) and > 50 years (n = 18/16). Cognitive levels are profoundly (MA ≤2, n = 13/11), low-severely (MA >2-≤3½, n = 17/19), high-severely (MA >3½-≤5, n = 22/19) and moderately mentally retarded (MA 5-8, n = 6/9).

RESULTS

Temperament

As to temperament a first question is: are they easy-going or troublesome? The temperament of mentally retarded fra X males is to be characterized as easier than that of the control subjects. This is particularly the case for men under 50 and for the moderately and severely mentally retarded. Looking at the TVZ-profile, we notice that in the fra X group several dimensions of the easy-difficult temperament factor scored higher (decile 7-8) than the median of the norm group of mentally retarded persons of the same cognitive level (decile 5-6) and than the median of the control group (general mood, intensity of reactions and appeasement).

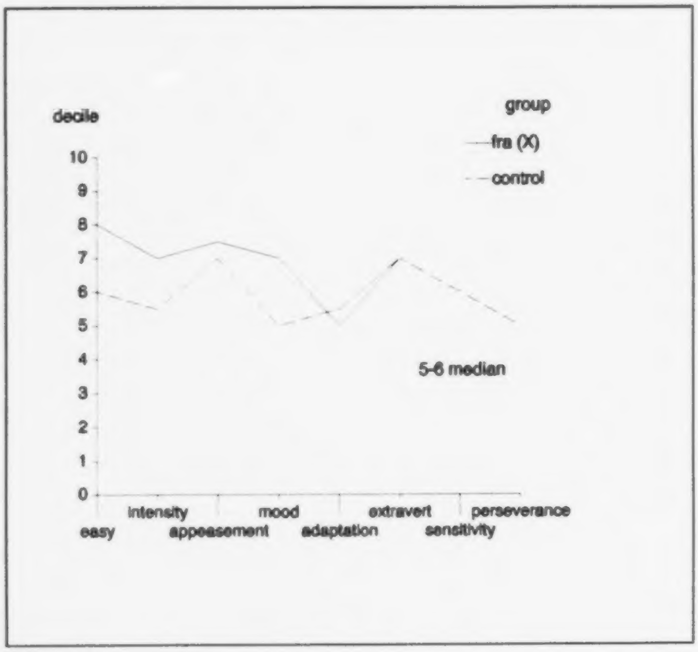

Figure 1

Comparison of the decile scores on the dimensions of the Temperament Scale for Mentally Retarded (TVZ) in the fra X (n = 58) and in the control group (n = 68)

The easier temperament of fra X males may, in the first place, be attributed to significantly higher scores on the dimension of general mood, i.e., in all age and level groups fra X adults have a more positive temper and are more susceptible to contact and attention offered by the staff than control subjects. Significantly differentiating items, each time in favor of the fra X group, are: being in a good morning mood, having a general good temper, giving a cheerful and optimistic impression, being satisfied and only sporadically grumbling, feeling happy and being friendly. In the second place the higher score in the dimensions of intensity and appeasement also contribute to the easier temperament, although not in a significant way for the whole fra X group. An interaction-effect for appeasement ($F(3,108)\ 3.56$; $p = 0.01$) indicates that only the moderately and the low-severely mentally retarded males are easier to divert from intense or nasty behavior than the control group; the profoundly and the high-severely mentally retarded on the contrary are less so. Fra X males can be differentiated from the control group in that they are less inclined to continue forbidden behavior. Both in the fra X group and in the control group, the lowest scoring dimension for this factor is the tempo of adaptation to new or changed situations, objects or people, although it remains in the average range of the norm groups.

The second factor of temperament, openness to the environment, does not differ between the two groups. Fra X and control persons both are equally extravert and interested in the physical and social world, even to a greater extent than the norm groups, they are equally attentive, sensitive and responsive to environmental stimuli and they have a similar, but rather low level of concentration and perseverance in activities. Only two concrete items are more prominent in the fra X group: they are sooner distracted when listening to music or to a story and show a greater interest in incidents involving somebody else (e.g., when someone is hurt).

GENERAL LEVEL OF SOCIALLY MALADAPTIVE BEHAVIOR

Concerning the general level of verbal or physical aggression or other forms of socially maladaptive behavior, the majority of both groups (62.1% - 68.97%) is found in the range of 1 standard deviation around the mean of the norm groups of mentally retarded persons from the same age in residential institutions and day care centers (SGZ-total score 5.25 -7.25). Typical for the fra X group, in contrast to the control group, is that a heterogeneous or mixed group of maladaptive behavior score significantly higher than either verbal ($t\ 2.35$; $p = 0.02$) or physically aggressive behavior directed to others ($t\ 2.65$, $p = 0.01$).

CHARACTER

Table 1

Mean (standard deviation) of raw scores or standard scores for TVZ, SGZ, ABC and ABS subscales in the fra X (n = 58) and in the control group (n = 58)

Subscale	Fra X	Control	t	p
TVZ-mean raw scores				
Openness to the environment	23.83 (4.19)	23.88 (5.01)	-0.05	0.96
-sensory sensitivity	27.12 (5.51)	26.22 (6.15)	0.83	0.41
-perseverance	21.83 (5.06)	22.77 (6.16)	-0.90	0.37
-extravert ability	22.55 (6.26)	22.64 (6.33)	-0.07	0.94
Easy-difficult temperament	48.07 (14.28)	42.67 (13.97)	2.06	0.04*
-tempo of adaptation	30.22 (6.91)	31.00 (6.87)	-0.60	0.54
-intensity	24.46 (6.14)	26.27 (6.32)	-1.57	0.12
-general mood	32.41 (4.05)	29.93 (4.46)	3.14	0.002**
-appeasement	25.24 (4.96)	23.79 (4.99)	1.57	0.12
SGZ-total standard score (μ 6,25, σ 1,25)	6.07 (1.30)	6.03 (1.25)	0.16	0.87
SGZ-verbal standard score	06.36 (1.20)	05.91 (1.37)	1.89	0.06
SGZ-aggressive standard score	06.32 (1.19)	06.22 (1.26)	0.41	0.68
SGZ-mixed standard score	05.91 (1.24)	06.14 (1.17)	-1.03	0.30
ABC-mean raw scores				
total score	28.09 (21.41)	21.86 (20.22)	1.61	0.11
sensory	3.40 (4.47)	2.60 (3.89)	1.02	0.31
relating	8.52 (7.33)	5.93 (6.71)	1.98	0.05*
body and object	5.72 (6.13)	3.95 (4.83)	1.73	0.08
language	6.53 (5.04)	5.00 (5.03)	1.64	0.10
social and self-help	3.91 (4.69)	4.45 (4.46)	-0.63	0.53
ABS-mean raw scores				
violence and destructiveness	3.91 (5.53)	3.88 (4.47)	0.04	0.97
antisocial behavior	6.22 (6.25)	6.00 (6.57)	0.19	0.85
rebellious behavior	6.19 (6.95)	4.83 (4.61)	1.24	0.22
untrustworthy behavior	1.52 (2.46)	1.14 (2.16)	0.88	0.38
withdrawal	3.55 (3.98)	3.71 (4.78)	-0.19	0.85
stereotyped behavior/odd mannerisms	3.46 (3.20)	2.41 (3.42)	1.71	0.09
inappropriate interpersonal manners	1.43 (2.74)	1.26 (1.84)	0.40	0.69
unacceptable vocal habits	3.05 (3.08)	1.96 (2.34)	2.14	0.03*
unacceptable/eccentric habits	3.72 (4.73)	2.95 (3.17)	1.04	0.30
self-abusive behavior	1.07 (1.76)	0.91 (1.66)	0.49	0.63
hyperactive tendencies	2.24 (2.64)	1.52 (2.14)	1.62	0.11
sexually aberrant behavior	1.00 (2.36)	0.71 (1.40)	0.81	0.42
psychological disturbances	5.48 (6.43)	4.77 (4.26)	0.70	0.49

* $p < 0.05$

** $p < 0.01$

AUTISTIC-LIKE FEATURES

Mentally retarded fra X adults have a somewhat higher general level of autistic behavior than the control subjects. Although not significant for the group as a whole, this is most striking in the age group under 50 and with the moderately and severely mentally retarded.

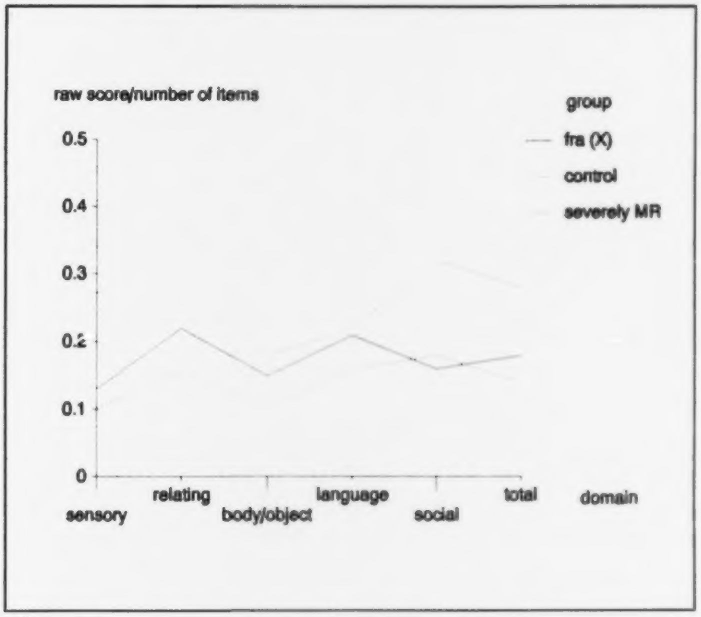

Figure 2

Comparison of the raw scores (in each subscale corrected for total number of items) for the dimensions of the Autism Behavior Checklist in the fra X (n=58) and in the control group (n=58)

As can be seen in the ABC-profile, the fra X group social relations (domain 2) and language (domain 4) are most autistic-like. Personal and social self-help skills (domain 5) is the only domain which is scored lower in the fra X than in the control group. 4/58 (6.9%) fra X adults and one (1.7%) control subject exceeded ABC-score 67, a high probability cutoff point for the classification of autism.

Firstly, the entire fra X group shows significantly more autistic features in connection with social interactions. Differentiating items are that more fra X males actively avoid eye contact (53.45% - 25.86%, p = 0.004), resist being touched or held

(44.83% - 22.41%, p = 0.01) and are stiff and hard to hold (34.48% - 12.07%, p = 0.008). For two other domains ABC-scores depend on an interaction between group and level of mental retardation: only severely mentally retarded fra X males have a more autistic body and object use (F(3.108) 2.95; p = 0.04) and more autistic personal or social self-help skills (F(3.108) 3.39; p = 0.02) than the control group; the others on the contrary are less so. The way in which fra X males deal with objects and use their own body is characterized by hand flapping (34.48% - 8.62%, p = 0.002) and insisting on keeping certain objects close by (32.76% - 27.59%). Concerning personal self-sufficiency, all subjects react strongly to changes in environment or routine (25.86% - 24.14%) and are frequently unaware of surroundings or oblivious in dangerous situations (25.86% - 20.69%). Fra X and control subjects have comparable scores on the domains of language and sensory sensitivity. The only differentiating language item is that more fra X males echo questions or statements made by others (36.21% - 12.07%, p = 0.005). Repeating sounds and words (34.48% -22.41%) or phrases (39.66% - 29.31%) over and over, is seen in a greater proportion of fra X males, without being statistically significant. The total ABC-score depends on age (F(2.110) 6.25; p = 0.003), but not on level of cognitive function. The youngest subjects under 35 have significantly more autistic-like behavior than the two older age groups.

OTHER TYPES OF SOCIAL DISTURBANCES

In the ABS-profile the negative peaks with a median percentile score higher than 80 relating to the norm groups of mentally retarded with the same age are for the fra X group to be found in the domains of automutilation (Pc83), stereotype of movements and odd mannerisms (Pc85), unacceptable vocal habits (Pc90) and hyperactive tendencies (Pc90). For the control group there are no such peaks. The behavioral difficulties that are least frequently observed run parallel in both groups: psychological disturbances (Pc65-63), untrustworthy behavior (Pc65) and violent or destructive behavior (Pc63-65).

Apart from the profoundly mentally retarded, control subjects use a lot more inconvenient and inappropriate verbal language (SGZ-verbal score) directed at others (F(1.108; 4.21; p = 0.04). This is manifested in that they accuse, abuse and trouble others more frequently. Similar, but not significant discrepancies are seen in the higher proportion of control subjects teasing and gossiping about others (43.10% - 58.62%) or being annoying by continuously bothering the staff either verbally or physically (29.31% - 46.55%). On the other hand, unacceptable vocal habits that are not immediately directed to others, but include disturbing vocal or speech habits, score on average higher in the fra X group, on all ages and cognitive levels. Examples of this seen in a significantly greater proportion of fra X males (82.76% - 60.34%, p = 0.01) are: talking and yelling loudly at others, laughing inappropriately, repeating words and phrases over and over and mimicking others' speech. These vocal characteristics are more prominent in the younger age group under 35 and diminish when the subjects grow older (F(2.110) 4.57; p = 0.01).

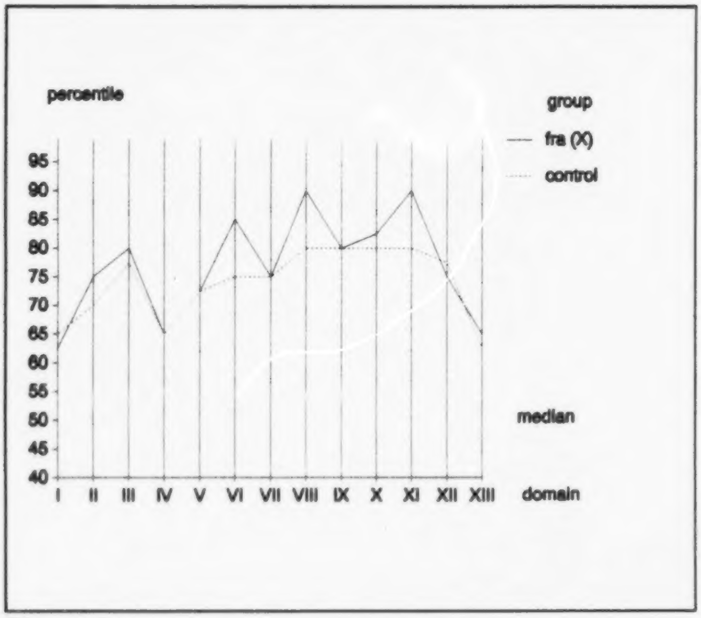

Figure 3

Comparison of the percentile scores for the domains of the Adaptive Behavior Scale in the fra X (n=58) and in the control group (n=58)

Legend to Figure 3: I. violent and destructive behavior; II. antisocial behavior; III. rebellious behavior; IV. untrustworthy behavior; V. withdrawal; VI. stereotyped behavior and odd mannerisms; VII. inappropriate interpersonal manners; VIII. unacceptable vocal habits; IX. unacceptable or eccentric behavior; XI. hyperactive tendencies; XII. sexually aberrant behavior; XIII. psychological disturbances.

Significantly more fra X persons, that is almost 80% (79.31% - 51.72%, p = 0.003) have some stereotyped behavior and these occur on average in a significantly higher frequency than in the control group (t 2.02; p = 0.04). Next to other aspects, we observed in the first place that the hands were constantly in motion and that they were waving or shaking parts of the body repeatedly. Stereotypes of movement and/or peculiar mannerisms are found especially with the profoundly mentally retarded (F(3.108) 3.46; p = 0.02) and in the younger age group under 35 (F(2.110) 3.72; p = 0.03). The fact that a significantly higher proportion of fra X males (56.9% - 36.21%, p = 0.04) show eccentric behavior in comparison to the control subjects, can be mostly explained by the already mentioned tactile defensiveness: one does not want to be touched and even screams when touched. Sleeping difficulties are signalized less frequently (t 2.49; p = 0.01) and in a smaller group of fra X males (8.62% - 20.69%) compared to the control group.

Next to these significantly discriminating characteristics, some types of behavioral disturbances are observed in more than 50%, but in an equally large percentage of fra X and control persons. Hyperactivity (fra X 63.79% - control 46.55%) with busy and irritated behavior (63.79 - 56.90%) is seen in almost 2/3 of the fra X group. Being shy (60.34% - 51.72%) and separating themselves from the group (46.55% - 39.66%) are in the course of the above mentioned autistic relational features. Some negative aspects of the direct contacts with others are threatening or being physically violent (51.72% - 58.62%) and bossing or manipulating others (53.45% - 46.55%). Many subjects in both groups react very poorly to criticism (68.97% - 72.41%) and to frustration (65.52% - 63.79%). Frequently mentioned examples of rebellious behavior are not complying with requests or orders belonging to the task-field (65.52% - 46.55%), ignoring regulations or regular routines (51.72% - 50.00%), resisting instructions, requests or orders (63.79% - 60.34%) and misbehaving in group settings (50.00% - 37.93%). Finally, self-injury, mostly in the form of biting oneself, is found in half of the fra X group (50.00% - 32.76%).

DISCUSSION

The aim of the present study was to delineate the consistency, the specificity and the age or cognitive level dependency of social-emotional features concerning temperament and character of adult men with fra X syndrome. We, therefore, performed extensive behavioral observations in a group of 58 mentally retarded fra X adults, aged between 21 and 67 years, comparing the results to a control group of 58 fra X negative mentally retarded subjects, matched for chronological age, cognitive level (mental age), living situation and period of institutionalization.

The fra X males' temperament is typical and distinct from the control group due to a very positive temper and openness to social contact and attention. In addition, moderately and severely fra X mentally retarded are easier to divert from intense or negative behavior. Most discriminating discrepancies in temperament dimensions are between the scores for general mood, on the one hand, and for perseverance or tempo of adaptation on the other hand, which are positive for the fra X group, but negative for the control group. The positive temperament of fra X males is in contrast to the observations that they may have some difficulties in coping with greatly or rapidly successive changes in situations. Fra X males also cannot pay attention for a long time and give up tasks easily. Although an easy temperament in itself is very positive personality trait, Blok (1989) offers a word of caution in that "easy-going" people are easier to be overlooked, because they are not very assertive and more contented, and could be overrated, because they reach an activity level in home and at work that is high in comparison to their cognitive abilities.

The average level of verbal and physical aggression and mixed forms of social disturbances corresponds to the norm groups of mentally retarded persons in residential institutions and day care centers for the fra X as well as for the control group. This is in contrast with the findings of Gielen et al. (1988) who observed higher levels for all three SGZ-subscale scores in a group of 20 moderately and severely mentally retarded fra X males compared to a control group. In the study of Gielen et al. (1988) it is especially the items of auto-mutilation, busy and irritated behavior, verbally or physically annoying behavior, stereotyped sounds and language and separating themselves from the group that are contributing to the high SGZ-scores in the fra X group. Although some of these were frequently mentioned in more than half of our fra X group, none was significantly discriminating from the control group. Dykens et al. (1988) reported that general levels for maladaptive behavior were lower for 12 mentally retarded fra X adults than for males with non-specific mental retardation and could not define a specific profile of socio-emotional function.

We have found conclusive evidence for a specific and differentiating pattern of characteristic behavior difficulties in a large group of mentally retarded fra X males. Autistic-like features, eccentric peculiarities such as tactile defensiveness, stereotypes and disturbing but not anti-social vocal habits are more frequent and/or more intense in the fra X group. On the other hand the social function of their verbal communication is in general more appropriate and they show less problem behavior concerning sleep. Although most of these social characteristics are striking in the fra X group as a whole, frequency and/or intensity of autistic-like, stereotyped and vocal features are also determined by age, being more prominent in the younger age group under 35 years. Apart from the motor stereotypes or peculiar habits, we could not confirm that social-emotional behavior is dependent on the severity of mental retardation.

Some authors (Hagerman, 1987, 1989; Reiss & Freund, 1990) have raised the possibility that communicative and social behavioral characteristics in the fra X syndrome could be related to sensory integration deficits. According to these authors, fra X males are hypersensitive, easily confused and overwhelmed by environmental or internal stimuli and have difficulties with sensory (auditory, tactile) processing and especially with sensory motor integration. Neither the results on the ABC-domain of autistic sensory characteristics nor the TVZ-temperament dimension of sensory sensitivity support these theories.

Autistic-like characteristics were frequently noted as discriminating fra X adults from other subgroups of mentally retarded adults: gaze aversion and repetitive body movements (Cohen et al., 1988, 1989, 1991; Dykens et al., 1988), perseveration, echolalia, talking to oneself, jargon and inadequate nonverbal communication (Wolf-Schein et al., 1987) and palilalia (Ferrier et al., 1991). The most important question is not whether fra X adults are autistic or not, but rather which concrete behavioral difficulties remind us of the association of fra X with the clinical autism syndrome. The results of this study suggest that a majority of fra X males has a specific subset of behavior items, which belongs to the autistic spectrum. Fra X adults' social function is characterized by avoidance of eye and tactile contact in social interactions, by stereotyped motor movements, and extreme attachment to objects, situations or fixed routines and by echolalic and repetitive language patterns. The same fra X subset of social difficulties was mentioned by Einfeld et al., (1989). Reiss & Freund (1990) supplemented their description with abnormal peer interactions and unusual reactions to specific sensory stimuli. It is important to notice that social interactions are not characterized by marked qualitative impairments in reciprocal social responsivity or empathy. Items that point to social indifference (e.g., do not reach out when reached for, not responsive to other people's facial expressions and feelings or 'looking through' people) are only seen in less than 10% of the fra X group.

The results of the present study objectively confirmed that the interaction style of fra X adults is determined by approach-avoidance conflicts, as suggested by Cohen et al. (1988, 1989), Fryns et al. (1984), Gielen et al. (1988), Hagerman (1991) and Wolff et al. (1989). There is a peculiar combination of autistic-like nonverbal avoidance behavior (shyness, avoiding eye contact, tactile defensiveness, separation) and a greater interpersonal sensitivity and caution for social contacts (susceptibility to attention, extravert ability and interest in the social world). Next to approach-avoidance conflicts, stereotypy of body movements, repetitive language and self-injuring behavior can also be used as mechanisms for expressing and coping with emotional tension and anxiety, especially in social situations (Bregman et al., 1988; Cohen et al., 1988; Sudhalter et al., 1990). Further study should assess whether social anxiety is caused by a primary deficit with attention and arousal regulation or rather by social-cognitive difficulties in organizing, interpreting, and subsequently, responding to complex social information.

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BEHAVIORAL ASSESSMENTS

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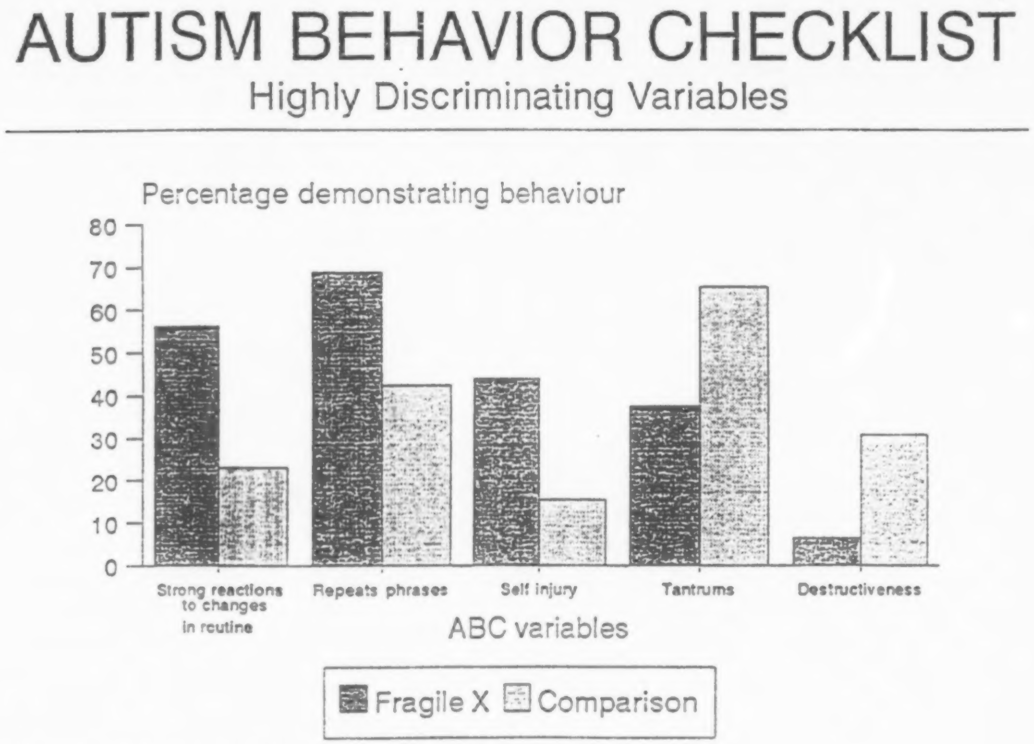
Behavioral checklists have been a favorite tool of psychologists for some time. They have been especially helpful with behaviorally deviant populations because they are easy to administer and are non-intrusive. A caretaker or parent is the informant, thus information is readily available.

Unfortunately, many behavioral checklists do not appropriately describe the behavioral features of the fragile X male. Many investigators have attempted to quantify autistic features in a fragile X sample (Borghgraef et al., 1987; Cohen et al., 1988; Einfeld et al., 1989) in order to better define the syndrome. Behaviors of fragile X males are consistent with autism more than any other behavioral sub-group.

In an attempt to develop a checklist more appropriate to screen for fragile X, I utilized the Autism Behavior Checklist (ABC) with 197 males who were cytogenetically evaluated for the fragile X syndrome.



Sample sizes: Fragile X 32, Comparison 26

Behavioral Assessments

A control group of 88 (cytogenetically negative) and an experimental group of 109 (cytogenetically positive) were compared. The results revealed the following:

1) Three behavioral descriptors were significantly different in the fragile X population from the control group:

A.	Strong reactions to changes in routine/environment	$p<.021$
B.	Flaps hands	$p<.002$
C.	Repeats phrases over and over	$p<.03$

2) Two symptom areas were significantly different in the fragile X sample from the control group:

A.	The Sensory symptom area	$p=.001$
B.	The Relating symptom area	$p=.011$

3) The total ABC score differed significantly in the fragile X sample from the control group:

A.	fragile X	mean = 50.86
B.	control	mean = 60.70

4) Age was a positive predictor of responses in the fragile X sample (younger children had more symptoms than adults):

A.	Sensory symptom area	$p<.008$
B.	Relating symptom area	$p<.017$
C.	Total ABC score for fragile X	$p<.04$

5) Five behaviors correlated with percent fragility:

A.	"pronoun reversal"	$r(105)=.2174$, $p=.026$
B.	"not reaching out when reached for"	$r(105)=.2711$, $p=.005$
C.	"severe temper tantrums"	$r(105)=.2246$, $p=.021$
D.	"not waiting for needs to be met"	$r(105)=.2264$, $p=.02$
E.	"feeling, tasting, or smelling objects"	$r(105)=.3164$, $p=.001$

DISCUSSION

This study confirms some similarities between fragile X and autistic behavior, however, without a significant causal relationship. This study contributes further to the behavioral phenotype of fragile X. Three behavioral features are demonstrated by fragile X subjects and are significantly different from controls. Additionally, two symptom areas, sensory and relating, differed significantly in fragile X males.

These findings are supported by many observations. A number of clinicians have reported that fragile X individuals relate abnormally to sensory input. Fragile X males typically exhibit these autistic-like behaviors when they are overstimulated. The autistic behavior is less constant in the fragile X sample and not reported as frequently on the checklist. The over-exaggerated sensory symptoms may very well be associated with anxiety. The results of this research further support the notion that there exists core features in the fragile X population which co-vary with the gene in such a way to define a behavioral phenotype.

Scores from the relating symptom area support other research data. Cohen et al. (1988) found that fragile X subjects related differently to strangers than familiar family members compared to their autistic counterparts. In addition, the relating symptoms were inconsistent and often anxiety driven. If this is true, it would help explain the "approach-avoidance" phenomenon often observed. The additional finding of younger boys being more aggressive than older males confirms earlier reports that adult fragile X males seem to improve behaviorally and are easier to manage with time. The next challenge is to use this information to formulate treatment plans and develop intervention strategies.

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BEHAVIORAL ASSESSMENTS

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Preliminary data is presented from a United Kingdom based study of Autism Behavior Checklist ratings for 32 children cytogenetically confirmed as having fragile X syndrome. A comparison group consisted of 26 children cytogenetically evaluated in the same genetic units but found to be negative for fragile X syndrome. Chronological age and developmental level distributions were not significantly different.

There was no significant difference between the two groups on their total ABC scores. However, the distribution of total scores for the two groups demonstrates potentially important variations. Firstly, it was very rare for fragile X children to show few autistic features, while comparison children more often did. Secondly, the most common scores for fragile X children were somewhat lower than those for the comparison group. Thirdly, there is a small but important number of children with fragile X syndrome who have quite high scores. An interpretation of these trends could be that children with fragile X syndrome tend to have a modest number of autistic-like features, with a small subgroup demonstrating typical autism. Comparison children with idiopathic mental retardation show greater variation including a small group with few autistic features and a greater number showing slightly more features than the average child with fragile X.

Certain checklist variables discriminated highly between the two groups. Three such variables were more common in fragile X children: strong reactions to changes in routine (p=0.02), repeats phrases (p=0.08) and self-injury (p=0.04). Two were more common in the comparison group: tantrums (p=0.06) and destructiveness (p=0.03).

These provisional results are consistent with similar findings from the United States suggesting it is more common for children with fragile X syndrome to show a number of autistic-like social impairments rather than typical autism.

Furthermore, certain of these impairments are frequently observed and are useful in distinguishing children with fragile X from other children with similar chronological ages and developmental levels. These features are important in the development of diagnostic checklists based on behavioral attributes. They also focus remedial intervention strategies on common areas of developmental and behavioral difficulty.

PSYCHOPHARMACOLOGY AND THE FRAGILE X SYNDROME

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My research includes hyperactivity/attention, brain injury and the general problem of psychopharmacology for mentally retarded people (Gualtieri, 1990). I have always seen a large number of autistic patients, because Project TEACCH, an autism program, is in our town (Gualtieri et al., 1987; Gualtieri, 1991). The fragile X syndrome is a relatively new interest. So, I have only a small amount of data to present. It is possible, though, some insights from the broader field of neuropsychiatry will be useful, or at least of interest. I propose to talk about some new developments in psychopharmacology, based on the work of our research group in Chapel Hill.

HYPERACTIVITY

The treatment of attention disorders and hyperactivity in children and adults was our earliest research interest, and we published several papers about the neuropsychological effects of stimulants and related drugs (Gualtieri et al., 1985; Gualtieri, 1991). It was the "frontal lobe hypothesis" of stimulant drug effects that led to our current interest in brain-injury patients, many of whom, of course, have frontal lobe injuries (Gualtieri & Hicks, 1985). Brain injury patients frequently have problems with inattention, impulsiveness, excitability and poor memory, and we learned that many of them respond favorably to low doses of psychostimulant drugs, like methylphenidate (Ritalin) and amphetamine (Gualtieri & Evans, 1988).

We also learned, to our dismay, that the most severe brain injured patients did not do well at all with stimulants. Beneficial effects were fleeting, and stimulant-induced neurotoxicity came on after only a few doses. The fact, however, that some degree of improvement was possible, even for a few hours or days, meant the patients were capable of improving, if only we could find the appropriate drug regime. After a good deal of trial-and-error, we learned that amantadine (Symmetrel) worked very well for patients who had severe traumatic brain injuries; for example, for agitation and hyperactivity during the stage of coma recovery, and for disinhibition, impulsiveness, abulia and anergia in chronically impaired patients (Gualtieri et al., 1989). There was a pattern to the effects of amantadine compared to the effects of the psychostimulants: patients who had relatively mild injuries responded best to the latter, while patients who had severe injuries, with much more extensive brain damage, did better when they were treated with amantadine. The explanation for this contrast is an example of the complexity of psychopharmacologic treatment.

Amantadine and the stimulants are both "dopamine agonists," that is, they both promote dopamine neurotransmission. But different behavior at the synaptic level accounts for the

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difference in their clinical activity. Stimulants cause the presynaptic neuron to release dopamine, while amantadine exercises its effect (probably) on the post-synaptic dopamine receptor. Therefore, stimulants require an intact presynaptic neuron to exert a therapeutic effect. Amantadine has no such requirement. It seems to stimulate the post-synaptic neuron directly. Therefore, it is effective for patients who have had brain stem damage, with impaired function at the level of the presynaptic dopaminergic neuron.

The fact that the clinical status of the patient would determine which dopamine agonist works best was a new finding, so we decided to test its salience to the problem of developmentally handicapped people, who had had a similar pattern of bad luck with stimulant drugs. Although hyperactivity is an extremely common problem in mentally retarded children, especially autistic children, stimulants have never been very effective as treatment. Hyperactive kids who do best on stimulants tend to be high-level (i.e., high IQ, few co-morbid problems). Occasionally a mildly retarded child will respond to stimulants. A moderately retarded child, rarely. A severely retarded child, virtually never. It is ironic the children who need the treatment the most are the least likely to respond favorably to stimulant treatment. It seems there is a direct correlation between IQ and one's chance of a favorable response to stimulant drugs.

The solution, as you may have guessed, is amantadine. In a paper published in 1991, we reported the drug had a 50% response rate in hyperactive, mentally retarded children and adults (Chandler et al., 1991). The doses were low-to moderate, from 25 mg bid to 100 mg tid, and the side effects were no more troublesome than the side effects of stimulants in higher-level patients. Since then, we have had good luck in a few clinical cases, by adding low doses of methylphenidate to low doses of amantadine; our current series includes some children with the fragile X syndrome.

Amantadine is not a cure-all or a silver bullet, but it is a safe option to try for mentally handicapped children who are extremely hyperactive and disorganized, inattentive and impulsive. Its effects are apparent within a few days, and there is no need for special monitoring, for instance, blood levels. Similar to stimulants, there are no adverse interactions with antiepileptic drugs, and low doses may actually raise the seizure threshold (Shields et al., 1985). There are no particular risks to long term treatment. It is certainly preferable to some of the usual (bad) alternatives, like neuroleptics. If it works, the effects are dramatic.

As a psychotropic drug, amantadine is in a class with the stimulants, folate, pyridoxine, buspirone and clonidine. If the clinical indication is present, the drug is worth a try. There is very little risk in trying. If side effects occur, they will disappear soon after the drug is discontinued.

SELF-INJURIOUS BEHAVIOR

For several years, we had a research project concerned with the pharmacologic treatment of self-injurious behavior (SIB). This is an extraordinary disorder of mentally retarded people; studies have demonstrated the occurrence of SIB in no fewer than 13% of the residents of state schools (Schroeder et al., 1980).

Our first group of research subjects were mentally retarded adults with incredibly long histories of severe SIB; two of the patients, for example, had actually blinded themselves, and one developed *dementia pugilistica* from chronic head-banging (Gualtieri & Schroeder, 1989). It is rare, these days, to see such cases. One attributes the change to improvements in living conditions and developmental programming for retarded people, and to improvements in medical care. But ten years ago, there were many such cases.

It was gratifying, therefore, to report long-term success with low-dose neuroleptic treatment (oral fluphenazine, 1-6 mg/d), in a double-blind placebo-controlled study, especially in this difficult group of patients. We were encouraged to extend the study to a larger group of patients.

When we had begun our work with SIB patients, we followed the established convention, and defined SIB entirely in behavioral terms. That is, all patients with SIB (regardless of cause) were eligible for study. As long as their problem was sufficiently troublesome, and if it hadn't responded to behavior modification, they could be treated in the experimental protocol.

As our work progressed, however, we discovered the fallacy in this method. SIB patients were not a homogeneous group. Just as the patients were retarded on the basis of different etiologies, so the occurrence of SIB in different cases was probably related to different causes (Gualtieri, 1989).

After our research was funded by a grant from the NIH, we were able to spend more time with SIB patients, and we had the opportunity to learn why they were afflicted with this terrible disorder. For example, some patients were self-injurious because of seizure disorders that were treated insufficiently. Others were self-injurious because of the sedating properties of anti-epileptic drugs (AED). When they were taken off sedating AED's, or when they were switched over to the psychotropic AED, carbamazepine or valproate, their problem with SIB diminished considerably, and in many cases, it virtually disappeared. One SIB patient in our series had Tourette's Syndrome, and responded well to the appropriate treatment for that. There were several cases of gastro-intestinal reflux; their SIB responded to the usual GI treatments. One patient appeared to have a bipolar affective disorder (manic-depression), and responded well to lithium. Another cause of SIB was tardive akathisia, a neuroleptic drug side effect (Gualtieri, 1990).

In fact, it became harder and harder to recruit patients into the experimental SIB paradigms, because so many of them could be treated effectively, even before the study began, simply by attending to the venerable method of differential diagnosis, and by applying the appropriate medical treatment on that basis (Gualtieri, 1989).

We learned that SIB is not a single, unitary behavioral disorder. It is, rather, a symptom that may originate in many different underlying conditions. Treatment for SIB, without a thorough medical/neuropsychiatric diagnostic evaluation, is necessarily symptomatic, like treating a fever with aspirin. It is possible this accounts for the relative failure of putative "treatments" for SIB over the years.

In medicine, treatment may be "symptomatic" -- aspirin for a fever, codeine for a cough -- or it may be "rational," that is, specifically targeted at the underlying medical cause of the symptom. For a problem like SIB, especially in adult patients, an intelligent neuropsychiatric diagnosis may guide treatment in a rational way.

Unfortunately, an accurate medical or neuropsychiatric diagnosis is not always possible. There are still many SIB patients whose problem cannot be explained within the established medical categories. Unhappily, this is most often the case for children. Neuropsychiatric disorders are not, as a rule, well differentiated in children. An autistic child who is hyperactive and self-injurious or aggressive may develop epilepsy during his adolescent years. When the seizures are controlled with appropriate medications, the behavior problems may subside as well. Another child, with the same pattern of behavior problems, might ultimately prove to have an affective disorder, and thus respond favorably to antidepressants or lithium. It is extremely hard, if it is possible at all, to make the diagnosis of epilepsy before the child has ever had an overt seizure, or to diagnose an affective disorder if the symptom-picture is disorganized and non-specific.

There remain at least a few cases, therefore, where the elegant process of differential diagnosis is unavailing. One is forced, then, to resort to "symptomatic" treatments. I shall discuss the symptomatic treatments for SIB and for aggression later.

THE LESCH-NYHAN SYNDROME

The search for a "rational" treatment for SIB that guided our research efforts was based on a truly extraordinary disorder of mentally retarded people, the Lesch-Nyhan Syndrome (LNS), a genetic deficiency in the enzyme HGPRT. People with the LNS have, among other problems, a compulsion to self-injure in the most horrible ways. Because of their tendency to self-mutilate, many patients have to have their teeth removed. Even though the LNS is a comparatively rare medical disorder, the severity of the clinical symptoms have made it a major target for neurobiological research.

Research has actually demonstrated a very clear relationship between the specific genetic abnormality in LNS, a specific enzyme deficiency, and specific deficiencies in neurotransmitters in particular regions of brain (Lloyd et al., 1981). This research is based on post-mortem studies of human beings. Animal models have been developed for the LNS and the syndrome of compulsive self-mutilation has been reproduced in laboratory animals (Criswell et al., 1986). In animals, the behavior may be interrupted by administering certain psychoactive drugs. This is a dramatic example of how experimental neurobiology can enhance our understanding of a mental disorder in human beings. Our research into the treatment of SIB was based on a theoretical model of the LNS, the dopamine D1 supersensitivity model. That is why we chose the neuroleptic fluphenazine (prolixin), because of its supposed antagonism of the D1 dopamine receptor.

At the time, we were impressed at the success of fluphenazine, and we felt that the D1 model was supported, if not affirmed, by our results. Treatment with fluphenazine, however, is not without drawbacks. The problems of tardive dyskinesia and tardive akathisia, as well as the risk of the neuroleptic malignant syndrome, have diminished the potential utility of this treatment.

I should explain the salience of these remarks to the clinical problems of people with the fragile X syndrome (fra X). We are all excited by the recent definition of the FMR-1 gene, and we can anticipate the same development of neurochemical and neuroanatomic models to demonstrate how the genotype is translated onto the phenotype; that is, how the genetic abnormality actually results in developmental delays and behavioral aberrations.

But if we remember the lesson of LNS, we will be spared the disappointment that inevitably comes of inflated expectations. For in LNS, preclinical research, for all its success, has not led to effective treatment for the behavioral manifestations of the disorder. In the fra X, it may also be a long time before advances in genetics and neurobiology are able to give us effective treatments. A "rational treatment" for the clinical manifestations of the fra X may be forthcoming, perhaps in a couple of years, or perhaps in ten or twenty. It is sometimes a long way from the laboratory bench to the clinic.

THE CORNELIA DE LANGE SYNDROME (CDLS)

The CDLS is a genetic disorder associated with mental retardation and behavior problems like hyperactivity, aggression and SIB. I have had the privilege to see a large number of CDLS cases, through my association with the CDLS Foundation, an organization of professionals and families similar to the National Fragile X Foundation.

My clinical studies of the CDLS have been published elsewhere (Gualtieri, 1990), so I shall just highlight the points most pertinent to the topic at hand. The most important issue is the misrepresentation of CDLS in the medical literature. CDLS patients have been described as autistic and compulsively self-injurious (Coleman, 1973; Greenberg & Coleman, 1973). Some of them are. But my survey demonstrated that only a small number of CDLS patients had severe behavior problems that warranted neuropsychiatric intervention. Most of the behavior problems of CDLS children were mild and self-limited.

When a genetic disorder, like CDLS, has a "behavioral phenotype," there is a risk of a concomitant "behavioral stereotype." That is, people may assume the behavioral aberrations that characterize some of the patients with the disorder are, in fact, characteristic of the disorder. In the case of CDLS, only the most extreme cases were written up in medical journals. So it was assumed that "autism" and SIB were universal, and inevitable.

The fra X also has a behavioral phenotype -- a range of phenotypes, in fact. But the potential for neuropsychiatric disorders that exists in the FMR-1 gene is not always expressed. It is important to remember there is a range of possible outcomes, and it is also important to avoid the groundless fears and unnecessary treatments that come of stereotyping human beings.

Much of the time, children with CDLS have difficult behaviors when their medical conditions, like GI reflux, are undiagnosed and untreated. CDLS kids are frequently given to laying supine, with back arched, and an angry expression on their faces. That has been interpreted as a psychiatric symptom: "bizarre posturing." In fact, it is a symptom of esophagitis.

Behavior problems in CDLS adolescents may grow severe, but that usually occurs if the children are treated inappropriately, for example, in classrooms that are developmentally unsuitable, or if their family circumstances are unstable or unduly stressed. Their problems may become persistent and intractable if they get the wrong kind of psychiatric treatment; for example, if they are institutionalized in large, barren and frightening state hospitals, or if they are "treated" with neuroleptic drugs. CDLS children seem to have a particular vulnerability to one of the most serious side effects of neuroleptic treatment, tardive akathisia. It is ironic the very drugs prescribed to control their difficult behaviors may actually increase behavior problems over the long run.

The reader will understand how this turns into a self-fulfilling prophesy. CDLS patients are supposed to be autistic, aggressive, self-injurious, by dint of biological necessity. Therefore, they should be treated with neuroleptic drugs and put away in institutions. Then they become withdrawn, aggressive, self-injurious. An oversimplification, perhaps, but one more example of behavioral stereotyping and the vicious circle it constructs. An example, also, of how psychiatric treatments, especially certain psychotropic drugs, may aggravate the problems they are meant to alleviate.

The last point I would like to make, while we are talking about the CDLS, is the importance of parent-based organizations like the CDLS Foundation and the National Fragile X Foundation. In my opinion, there is no development so encouraging in medicine than the organization of professionals and families in associations that advocate for patients, for treatment and for research (in that order). It is good for communication, for the dissemination of knowledge and for the development of new knowledge. Most important, it is essential for setting priorities, and keeping them straight. I have had experience with organizations that began as consumer-advocates but then were co-opted by professionals, who have the unnerving habit of using them to advance their own priorities. This has never happened in the CDLS Foundation, because the professional consultants have done a good job protecting their proper role: as servants to the patients and to their families.

Since I am a newcomer to the National Fragile X Foundation, I should refrain from preaching to you about where to put your priorities. May I suggest, however, that the strength of a patient-advocate organization is best measured by how effectively it serves patients, and only secondarily by how well it serves doctors.

TARDIVE AKATHISIA

Because I have mentioned the problem of tardive akathisia three times already in this paper, it is appropriate to digress from the topic of CDLS, and to define the term. Tardive akathisia is a side effect of long-term treatment with neuroleptic drugs, like haloperidol (Haldol) and thioridazine (Mellaril) (Barnes & Braudse, 1985). It is kin to tardive dyskinesia, a movement disorder caused by the same drugs (Gualtieri & Barnhill, 1988). Tardive akathisia is not a movement disorder, but a behavior disorder, and the symptoms are extreme restlessness and dysphoria (Gualtieri & Sovner, 1989). The symptoms usually emerge when the neuroleptic dose is reduced or after the drugs are discontinued. It may go on for months, even years, after complete drug withdrawal (Gualtieri et al., unpub).

The misery of akathisia may cause a patient to explode in fits of aggression or self-injury. The symptoms of akathisia may be misinterpreted, therefore, and read as "target behaviors" that warrant continued treatment with psychotropic drugs. The irony, then, is patients may continue on the very drugs that have caused the disorder in the first place.

The treatment of tardive akathisia is not very successful, and the best course is usually to refrain from neuroleptics and to wait for symptoms to subside on their own. That is not a sanguine prospect for a disorder that may last for years. The best "treatment" for tardive akathisia is prevention: avoiding neuroleptic drugs unless they are absolutely necessary, using judicious doses when they are needed, and finding alternative drug treatments whenever possible (Gualtieri, 1991).

As it happens, there appear to be some patients who are especially prone to neuroleptic side effects like tardive dyskinesia and tardive akathisia. One important risk factor is affective disorder. Patients whose psychiatric disorders are characterized by emotional instability are probably at particular risk for developing neuroleptic side effects like tardive dyskinesia/akathisia (Gualtieri, 1990). CDLS patients represent one such group, fra X patients may be another.

I suppose one could design a research project to test the hypothesis that fra X patients are especially vulnerable to neuroleptic side effects. Until that project is done, I would recommend a conservative approach to neuroleptic treatment. For the neuropsychiatric problems of fra X people, there are usually better alternatives than the neuroleptics.

THE TREATMENT OF AGGRESSION AND SELF-INJURIOUS BEHAVIOR

It is the opinion of many neuropsychiatrists that SIB and pathological aggression are very similar in terms of their differential diagnosis and response to treatment. That fact ought to render this extensive discussion of SIB less oblique, since aggressive behavior is probably more frequent in fra X patients than SIB. In fact, the two symptoms may often co-exist.

There is an algorithm around the treatment of SIB and aggression in developmentally handicapped individuals, including fra X patients. It assumes that the usual medical/neurological etiologies have been excluded, and that the patient is in a developmentally appropriate program, living in respectable circumstances. Common-sense approaches to behavior management have been tried, and they haven't been effective. Aggression and/or SIB occur on a regular basis. The problem is so severe that a normal life is impossible, and thus one is led to consider a trial of psychotropic drugs.

There is no single drug treatment for the problems of aggression and SIB. There are, rather, a number of alternative treatments. Several different drug trials may be necessary before a good result is achieved. By "good result" we mean this: the problem is reduced substantially, or clears entirely; the patient is not sedated or dull as a result of treatment; and the risks of long-term toxicity are minimal. A good result, according to these criteria, can be achieved in the large majority of cases.

If the problem of aggression and/or SIB occurs in the context of hyperactivity, disorganization, impulsiveness or excitability, one should consider a trial of stimulants and/or amantadine, as described in the section above.

If the problem occurs in the context of anxiety, panic and hyperreactivity, one may consider buspirone (5 mg bid to 15 mg tid), clonidine (0.1 mg transdermal patch), beta-blockers (e.g., inderal SA 80 mg/d), or low-to-moderate doses of antidepressants (e.g., trazodone 50 mg bid; imipramine 1-3 mg/kg/day). Combinations are possible: for example, methylphenidate with amantadine, dopamine agonists with clonidine or beta-blockers or buspirone, etc. Folate (5 mg bid) and pyridoxine (50-200 mg/d) may also be useful.

This approach to treatment is more likely to be taken for pediatric patients. In adolescents and adults, the problems of aggression and SIB usually occur in the context of what psychiatrists call "Organic Mood Disorder," a more serious and persistent problem, and one that calls for treatment with drugs more complicated than the ones listed. The treatment of organic mood disorders is usually with lithium, carbamazepine or valproate, singly or in combination. These three drugs require blood level monitoring, and the usual therapeutic levels are necessary to achieve optimal effect. The side effects of lithium, carbamazepine and valproate are formidable, but they can be prevented, or at least contained, if the physician is familiar with them. Nevertheless, the drugs are much more "expensive" in terms of the medical monitoring required, and their potential for serious side effects if monitoring is not done by an experienced physician.

Once again, drug combinations are possible for particularly difficult cases. Acetazolamide, buspirone and the dopaminergic drugs work well in combination with lithium, carmabazepine and valproate.

There is special interest in the serotonergic drugs for affective disorders in fra X patients. Fluoxetine (Prozac) has been a popular choice, and buspirone, too. Sertraline (Zoloft) is a new selective serotonin reuptake inhibitor that has a similar spectrum of activity to fluoxetine, but the dosing schedule is more flexible and the half-life of the drug is shorter. Side effects, then, are usually easier to control.

The serotonergic drugs are used in combinations, for some refractory affective disorders, as well as for severe aggression or SIB, when one drug is not sufficient. Combining serotonergic drugs is something that is best left to an experienced psychopharmacologist. It is not dangerous, but it may be a complicated business. In young patients, pre-treatment with L-tryptophan (not currently available in the USA) or with a serotonin-rich diet may be necessary to maximize the effects of serotonergic drugs.

Obviously, there is a role for neuroleptic drug treatment in fra X patients with schizophrenia-type symptoms, and whose problems with aggression and SIB occur in that context. But the neuroleptics are given to some troublesome long-term side effects, and they should only be used for psychotic symptoms. Drugs like haloperidol (Haldol) and thioridazine (Mellaril) are rarely recommended for behavior problems like hyperactivity or aggression.

The treatment of severe, "idiopathic" SIB has been the topic of several papers, and it is not something I should expand upon here. The most promising developments are the serotonergic drugs, usually in combinations; naltrexone, in doses from 50 to 200 mg/d; and low-dose neuroleptics (e.g., fluphenazine, 1-6 mg/d).

As I mentioned before, our experience with the fra X is limited. But the few terse paragraphs, foregoing, capture the large part of our psychopharmacology practice with developmentally handicapped individuals, for problems very similar to those of fra X individuals. Before I came to the Snowmass/Aspen conference, I polled my recent experience with fra X patients, along with that of my colleagues, Mark Hauser, MD, of Boston, and Mark Chandler, MD, in Chapel Hill. In the past year we had only eleven fra X patients who had behavior problems sufficiently severe to warrant psychotropic drug treatment. Their psychiatric conditions were those described above: hyperactivity, explosive rage attacks, aggression, SIB, oppositional behavior, etc. The psychiatric diagnoses were Organic Mood Disorder in nine patients, including all eight of the adults; and hyperactivity/disorganization for two of the three children.

Because this was a clinical survey, I can only report "good results": behavior problems resolved, affect stabilized, a normal life in school or at the workshop, no side effects, psychiatric visits only for routine drug maintenance. Carbamazepine was the key element in successful treatment for eight of the nine patients with mood disorder; the ninth patient had responded favorably to carbamazepine, but he developed a drug allergy, so valproate was used instead. In four of the patients, combinations were necessary: carbamazepine with buspirone (1), with valproate (1) and with neuroleptics (2). The two hyperactive children were managed on amantadine.

SUMMARY

I have tried to give the reader an overview of some of the newer developments in psychopharmacology, if not for the fra X patient, at least for neuropsychiatric patients who have problems that also occur, with substantial frequency, in the fragile X syndrome. It is impossible to describe all the tricks a psychopharmacologist uses to achieve that vaunted "good result," but I have described some of the principles and a few of the treatments. A more extensive review of this information, with all of the appropriate references, is published in a recent book (Gualtieri, 1990).

People often ask how to keep up with the psychopharmacolgy literature, especially as it pertains to special populations, like mentally handicapped people, short of scanning the psychiatric journals. It is hard to answer that question. It is ironic, that even if one were to scan the journals on a regular basis, one would find very little relevant information.

A great deal of what neuropsychiatrists do for developmentally handicapped people is extrapolated or inferred from other areas of research. The idea of treatment with dopamine agonists, like amantadine for example, has been developed from the neurology literature, especially traumatic brain injury. The use of serotonergics, especially in combination, is a method that began in clinics for patients with obsessive-compulsive disorder. Discussions of carbamazepine, valproate and lithium are scattered throughout the literature, but they are hardly ever summarized.

Physicians are advised to make clinical recommendations on the basis of information developed in controlled clinical trials. That may be a worthy goal, but it is neither attainable, nor is it entirely desirable. For example, the SIB treatment written-up most frequently is naltrexone, an opiate antagonist. If one were guided simply by the published literature, one might assume naltrexone were the treatment of choice for SIB. In fact, nothing could be further from the truth. When we surveyed psychopharmacology practice among several thousand residents of state schools across the country, in 1988-90, we discovered frequent recourse to naltrexone for SIB patients; the number of clinical responders was vanishingly small (Gualtieri, 1991).

Medicine may never get around to controlled studies for small, special populations, like CDLS children. And funding priorities will never satisfy all of the clinical research requirements for large populations, like the fra X. The exigencies of research mitigate against the publication of articles about psychotropic combinations, as a rule, even though clinicians are increasingly inclined to use drug in low-dose combinations. As the medical literature becomes increasingly "scientific," that is, more precise, it tends to avoid the ambiguities that constitute clinical practice. As scientific precision is attained through the careful study of relatively simple models, the tendency is to reduce complex clinical problems to manageable components. Precision, then, is achieved at the risk of irrelevancy.

The development of psychopharmacology for special populations depends of a broad base of knowledge and experience in neuropsychiatry. It also requires extensive communication among practitioners, and the summary collection of vast amounts of clinical information. New treatments for children and adults with fra X will probably not develop in the context of restricted, controlled research studies. They will develop out of the organization of information from diverse sources, the expansion of information clearing-houses (did it occur to you that the National Fragile X Foundation was an "information clearing-house"?), and perhaps the creation of computerized databases. Medical prejudice against "clinical anecdote" is based on the weakness of a single practitioner's opinion. The collective opinion of a large number of practitioners, organized in a patient-oriented association, may not be so weak at all. What it lacks in precision, it makes up for by its relevance.

NOTE TO THE READER: I have used the first person singular, for the most part, because there was only one of me presenting at the Snowmass/Aspen conference. When the first person plural was used, it was to refer to the work of our research team in Chapel Hill. My fellow authors are acknowledged in the reference section.

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SEROTONIN RECEPTOR SUBTYPES AND THEIR IMPLICATIONS FOR PSYCHIATRIC DISORDERS

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ABSTRACT

The understanding of psychiatric disorders and of brain structure and function is co-evolving (Preskorn, 1990). This paper will provide an overview of serotonin receptor subtypes, the brain functions subsumed by central serotonin systems, and the possible relationship between this neurotransmitter and human psychopathology,

The proposed number of serotonin receptors is rapidly expanding and includes four main families: 5-HT1, 5-HT2, 5-HT3, and 5-HT4. The 5-HT1 family is further subdivided into 5-HT1A, 5-HT1B, 5-HT1C, 5-HT1D, and 5-HT1-like (Hartig, 1989; Peroutka, 1988; Van Van et al., 1990). Two subtypes of 5-HT2 and three subtypes of 5-HT3 receptors have been proposed based on binding experiments. The existence of these subtypes have also been verified by differences in regional distribution, mediation of different physiological functions, linkage to different second messengers, and molecular structure. Three 5-HT receptors (5-HT1A, 5-HT1C, and 5-HT2) have now been cloned. These receptors belong to a G protein receptor class, whereas the 5-HT3 receptors belong to a ligand-gated ion superfamily (Hartig, 1989).

Central serotonin mechanisms have been postulated to be involved in numerous physiological (e.g., feeding, sleep, hemodynamics) and pathophysiological (e.g., anxiety, depression, alcohol abuse) processes. Medications which specifically interact with different aspects of this system can be clinically useful and can also serve as tools to aid in better defining the relationships between these apparently disparate conditions.

INTRODUCTION

What do alcoholism, anxiety, affective disorders, aggression, eating disorders, sleep disorders, and suicide have in common? Serotonin has been implicated in all of them. For this reason as well as others, it is the leading candidate for the title, "neurotransmitter of the 1990's." While we are early in "the decade of the brain," any neurotransmitter challenger will have considerable distance to cover to catch up.

Yet, the term "neurotransmitter" might well be a misnomer when applied to serotonin.

Neurohumor might be more accurate. The name serotonin itself means serum tonic. It could just as well have been called brain tonic. In terms of basic brain functions, serotonin mechanisms are important in feeding, fighting, mating, and sleeping.

How one neurotransmitter can play a role in such a diverse range of physiology and psychopathology is the subject of this paper. It will provide a historical review of developments in knowledge about serotonin, a summary of current understanding of serotonin receptor subtype, and the possible roles they play in brain function and psychopathology.

HISTORY OF SEROTONIN

Sjoerdsma and Palfreyman have divided the history of serotonin into four epochs: Primordial, Renaissance, Drought, and New Wave (Table 1) (Sjoerdsma et al., 1990).

Table 1
History of Serotonin Knowledge

Primordial Era (- 1953)

- Serotonin
  = serum tonic factor (Page)
  = enteramine (Erspamer)
- Key locations
  = brain, platelet, gut

Renaissance Era (1953-70)

- Serotonin synthesis, degradation, pharmacology
- Hypertension, carcinoid syndrome, mental illness

Drought Era (1970-79)

- SE precursors, L-tryptophan and 5-hydroxytryptophan, have antidepressant effects; whereas, NE precursors, L-dopa, does not
- PCPA but not AMPT reversed antidepressant effects of IMI and tranylcypromine
- SRIs have antidepressant effects

New Wave Era (1979 -)

- SE receptor subtypes
- Development of new molecular entities

During the first era, serotonin was isolated, identified, synthesized, localized, and named. These critical first steps in our understanding were completed only 40 years ago. By that time, we knew the serum tonic factor, "serotonin," and the smooth muscle contracting factor, "enteramine," were one and the same. Fortunately for the decade of the brain, "serotonin" won out over "enteramine" as the accepted name for this neurotransmitter. By the end of the first era, serotonin was recognized to exist in three principal location in the body: the serum (platelets), the gut and the brain.

During the renaissance era, the elementary biochemical processes important to serotonin synthesis and degradation were discovered. From this understanding, drugs were developed that could impact at different steps in serotonin metabolism. These drugs (and their effects) included: reserpine (transmitter release and depletion), monoamine oxidase inhibitors (block degradation), ergots and cyproheptadine (receptor antagonism), parachlorophenylalanine (block synthesis), and tricyclic antidepressants (block reuptake). Such agents then served as tools to understand the central functions subsumed by serotonin.

In the 1970's, the major activity was mapping the central serotonin system. This system was found to be one of the most diffusely organized projection systems in the brain (Descarries et al., 1990; Tork, 1990). It has two main components: a rostral and a caudal division. The rostral system has its cell bodies in the midbrain and the pons and projects towards the forebrain. The caudal system has its cell bodies in the medulla oblongata and projects to the spinal cord. The rostral system sends projections to virtually every upstream brain region including thalamus, hypothalamus, basal ganglia, limbic system and neocortex.

Via these anatomical connections, serotonin plays its role in the physiological and psychopathological processes listed at the beginning of this article. The question is how could such a diffuse system have any specificity? This question was made more perplexing by the anatomists' discovery that most serotonin fibers do not make specialized synaptic contact with the target neuron. Some ultrastructural neuroanatomists have suggested only 15 - 40% of serotonin fibers make specialized synaptic contact depending upon brain region being examined (Descarries et al., 1990). Instead, serotonin appears to be released from varicosities. Such an arrangement suggested that serotonin had more "tonic" than point to point transmission functions within the brain. Perhaps, serotonin would be better described as a brain "tonic," or at least a neurohumor, rather than as a neurotransmitter, the latter term being reserved for substances which are released into specialized synaptic clefts to have very localized effects. A possible answer to the question of specificity of action had to await the discovery of subtypes of serotonin receptors.

During the era of the neuroanatomists, the psychopharmacologists' interest in serotonin waned. Particularly in the United States, norepinephrine gained preeminence as the putative transmitter underlying affective and anxiety disorders. Nonetheless, three pivotal observations implicating serotonin in the pathophysiology of depressive disorders were made during this decade (Sjoerdsma et al., 1990; Shopsin et al., 1976; Delgado et al., 1990). First, serotonin (i.e., L-tryptophan, 5-hydroxytryptophan) not norepinephrine (L-dopa) precursors were reported to have antidepressant properties. Second, inhibition of serotonin but not norepinephrine synthesis was found to reverse the effectiveness of antidepressant agents. Third, the antidepressant efficacy of serotonin selective reuptake inhibitors was identified.

The second and current renaissance era was ushered in by the report of Peroutka and Snyder in 1979 of the identification of multiple serotonin receptors (Peroutka & Snyder, 1979). This work revived the interest in this concept which had first been raised by Gaddum and

Picarelli in 1957 (Gaddum & Picarelli, 1957). The present dizzying pace of understanding the diverse nature of serotonin receptor subtypes had to await the discovery of more sophisticated techniques.

RECEPTOR SUBTYPES

Over the last decade, the criteria for characterizing and hence defining receptor subtypes has continued to expand. At first, the suggestion of subtypes was primarily based upon identifying differences in ligand binding affinity. Now, the existence of receptor subtypes can be confirmed using a multitude of different but mutually supportive techniques.

While psychiatrists sometimes see their field as being unique in terms of continuous changes in their nomenclature systems, there are perhaps even more changes in the area of classifying serotonin receptor subtypes (Hartig, 1989; Peroutka, 1988; Van Van et al., 1990). The rapidity of our evolving understanding of these subtypes is such that what follows may be dated by the time it is printed. Yet, it should provide a reasonable overview to the area.

The "lineage" of serotonin (5-HT) receptors is now divided into four "families." These are: 5-HT1, 5-HT2, 5-HT3 and 5-HT4. Each of these families has several members (Table 2). These members have been characterized to varying degrees by their differences in ligand binding affinity, second messenger systems, regional localization, physiological functions and molecular structure. With regard to the latter, three subtypes of serotonin receptor subtypes have been cloned as of December 1990: 5-HT1c, 5-HT1a, and 5-HT2. The major distinctions between 5-HT1, 5-HT2 and 5-HT3 receptors are presented in Table 3 as well as some caveats about 5-HT1b and 5-HT1c receptors.

Table 2
Serotonin Receptor Subtypes

5HT1: A, B, C, D, E, -like
5HT2: A, B
5HT3: A, B, C
5HT4: ?

Just as psychiatrists have difficulty knowing how different Generalized Anxiety Disorder truly is from Dysthymia, serotonin researchers are uncertain sometimes whether a proposed subtype fits better into one family or another.

Table 3
5HT1 and 5HT2

- are different gene products
- have a significant degree of sequence homology
- belong to G protein-coupled receptor superfamily
- have common structural feature of seven membrane spanning segments

5HT3 Receptors

- belong to ligand-gated ion superfamily

Caveats

- 5HT1B may be rodent specific
- 5HT1C may belong to 5HT2 family based on molecular biology and pharmacology data

One important way to test varying hypotheses is to identify the regional distribution of these receptor subtypes (Table 4). Such localization can be accomplished through a number of techniques (Tork, 1990). The serotonin pathways themselves can be mapped by histofluorescence and immunohistochemistry. Within the terminal field of these pathways, serotonin receptor subtypes can be identified by autoradiography and more recently and more specifically by *in situ* hybridization histochemistry. In this latter technique, molecular cloning of the subtype has permitted the development of synthetic oligonucleotides complementary to selected sequences of the messenger RNA coding for the receptor. By staining the brain slides with the oligonucleotides, the cells expressing the gene which codes for the receptor subtype can be identified. Currently, this technique is possible only with three 5-HT receptor subtypes, but more are only a matter of time.

Table 4
Serotonin Receptor Subtypes and Regional Brain Distribution

Subtype	Localization
5HT1A	Limbic system
5HT1B and 5HT1D	Basal Ganglia
5HT1C	Choroid Plexus and Basal Ganglia
5HT2	Neocortex
5HT3	Limbic system - periphery link

Thus, in addition to receptor subtypes, the diffuse central serotonin system can have specific effects on brain function by regional localization of action (Descarries et al., 1990; Tork, 1990). Having different receptor subtypes and having them preferentially localized in specific brain regions can help us to understand how serotonin could be implicated in so many elementary physiological functions and distinct psychopathological states.

RECEPTOR SUBTYPES AND PSYCHOPATHOLOGY

What evidence is needed to causally link dysfunction in a central neurotransmitter or neurohumoral system such as serotonin to a specific behavioral disorder? Like Koch's postulates for bacteriology, similar criteria can be developed for the neuroscience of psychiatry (Table 5). These criteria require data from multiple types of studies to increase the confidence in the conclusions.

Table 5
Criteria to Relate Dysfunction in a
Neurotransmitter System to Psychiatric Illness

- Abnormalities in system exist in patients with the illness
  Example: CSF 5HIAA, 5HT2 binding
- Treatments which produce or correct the abnormal findings produce or correct the condition
  Example: ADs and 5HT2 binding
- Physiological evidence that the brain regions which contain the transmitter system have altered function during symptomatic episodes but not during remissions.
  Example: Positron emission tomography

Whereas a few years ago we would not have had the tools to attempt to tests such links, technological advancements have made it feasible. These techniques include: measurements of neurotransmitter or metabolite levels, brain imaging studies focusing on regions of interest, physiological measurements in response to provocative challenges (e.g., prolactin release in response to fenfluramine challenge), and the measurement of response to drugs with specific mechanisms of actions (e.g., azapirones). The latter two provide tests of the functional integrity of the transmitter system. These different approaches are mutually supportive rather than being duplicative. From such research, we can develop specific theories linking serotonin dysfunction mediated via specific receptor subtypes in specific brain regions to specific forms of psychopathology (Peroutka et al., 1989).

Such data already exists to varying degrees for certain forms of psychopathology. This data supports the development of testable hypotheses (Table 6). At this stage, these hypotheses are presented for their heuristic value. In addition to guiding future research, these hypotheses can provide a framework within which to utilize existing medications and to understand their effects in a given patient.

Table 6
Serotonin and Pharmacology Disease

Disease	Pharmacology
Alcoholism	5HT1A agonism, SRI
Anxiety	5HT1A agonism, 5HT2 antagonism 5HT3 antagonism, SRI
Depression	5HT1 agonism, 5HT2 antagonism SRI
Emesis	5HT3 antagonism
Food Intake	5HT1A agonism (+), SRI (-)
GI hypermotility	5HT3 antagonism
Hyperactivity	5HT1A agonism
Hypertension	5HT1A agonism
Impulsivity	5HT1A agonism (-), SRI (±)
Migraine (acute)	5HT1D agonism, 5HT1A agonism
Migraine (prevention)	5HT2 antagonism, SRI
Obsessions	SRI, 5HT1A agonism
Pain	5HT2 antagonism, 5HT3 antagonism
Schizophrenia	5HT3 antagonism
Sleep	5HT2 antagonism

It is beyond the scope of this paper to exhaustively review the data supporting the hypotheses presented in Table 6. Still, a brief review is desirable to provide a further basis for a discussion of the implications of this understanding for pharmacotherapy.

SUICIDE STUDIES

Low cerebrospinal fluid (CSF) concentration of 5-hydroxyindoleacetic acid (5-HIAA) in patients who make violent and impulsive suicide attempts has been one of the most consistent findings in biological studies in psychiatry (Asberg et al., 1987; Roy et al., 1989; Stanley & Stanley, 1988). Since 5-HIAA is the major metabolite of serotonin in the brain, this finding has been taken to reflect a deficiency (or at least a dysfunction) in central serotonin mechanisms in such patients.

This finding has been extended by the postmortem study of brains of suicide victims (Arango et al., 1990; Arora & Meltzer, 1989; Demeter et al., 1989; Lawrence et al., 1990; Mann et al., 1989; Meyerson et al., 1982; Stanley et al., 1982). In these studies, such patients have modest decreases in brainstem concentrations of 5-HT and 5-HIAA. In addition, an increase in 5-HT2 receptors has been reported in the neocortex of suicide victims. Such an upregulation would be an expected compensatory change to offset a relative deficiency of the neurotransmitter at such receptors.

Consistent with this explanation are the findings from animal studies in which the central serotonin system has been chemically or physically lesioned. Such lesions produce the same changes as reported above: decreased CSF 5-HIAA and brain 5-HT and increased 5-HT2 binding in neocortex.

DEPRESSION STUDIES

The vast majority of patients who commit suicide have major depressive disorders. Consistent with the above reports, there is evidence of dysfunction in at least a subset of depressed patients (Asberg et al., 1987; Mann et al., 1989; Coccaro et al., 1989; Meltzer, 1989; Robinson et al., 1898). The evidence includes: a diminished prolactin response to fenfluramine challenge in depressed patients, and the antidepressant effectiveness of several classes of drugs with specific serotonin mechanisms including reuptake inhibitors, 5-HT2 antagonists, and 5-HT1a partial agonists as well as the weak antidepressant activity of serotonin precursors. In fact, the serotonin theory of depression led to the first successful theoretically based antidepressant augmentation strategy, the addition of lithium carbonate to tricyclic antidepressants (de Montigny et al., 1981). Unfortunately, the multitude of actions of lithium severely restricts the value of this observation in terms of supporting the serotonin theory.

Additional evidence for a serotonin role in depressive disorders includes the observation that depleting central serotonin reverses the antidepressant effects of several types of antidepressant agents. The first report of this phenomenon came from Shopsin and colleagues who used parachlorophenylalanine to block serotonin synthesis (Shopsin et al., 1976). Subsequently, Delgado and coworkers have reported a similar phenomenon using a tryptophan free amino acid drink to acutely lower central serotonin levels (Delgado et al., 1990).

ALCOHOLISM

For some time, an inverse relationship has been known to exist between the integrity of central serotonin function and the consumption of alcohol (Baday & Evans, 1976; Wong et al., 1990). Selective serotonin reuptake inhibitors have been reported to reduce alcohol ingestion in both laboratory animals and in man (Wong et al., 1990). More germane to this discussion, chronic alcohol ingestion can produce changes in the brain reminiscent of the findings in violent suicide attempters and suicide victims: reduced CSF 5-HIAA and brain 5-HT in rodents, and decreased 5-HT transporter protein and increased 5-HT2 binding in the neocortex of alcoholics (Stanley & Stanley, 1988; Gross-Isseroff et al., 1988; Gross-Isseroff et al., 1990).

These findings are of particular interest since alcohol can induce an organic affective disorder that is sufficiently phenomenologically similar to major depressive disorder to represent a common difficult diagnostic problem (Schuckit, 1986; Preskorn & Goodwin, 1987). Alcohol is also a frequent complicating factor in suicide attempts and completions. The most common explanation is that alcohol reduces inhibition and thus makes an individual who is contemplating suicide more likely to act. The fact that it produces a pattern of changes in central serotonin parameters similar to that seen in violent suicide attempts, suicide completions and depressive disorders suggest another explanation. Alcohol may produce the same physiological changes in the brain which mediates these behaviors.

IMPULSE CONTROL

This area has perhaps the richest array of data supporting a serotonin link. The data includes first the fact that serotonin innervates regions in the limbic system which have been implicated in mediating species-specific aggression in animals and man (Peroutka, 1988; Sjoerdsma & Palfregman, 1990; Descarries et al., 1990; Tork, 1990; Peroutka et al., 1989). Second, lesioning of the serotonin systems in animals and man alters the threshold for aggressive behavior in animals and man (Asberg et al., 1987; Linnoila et al., 1989; Apter et al., 1990; Coccaro, 1989; Gray, 1982). Third, low CSF 5-HIAA has been found in violent prone individuals (Peroutka et al., 1989; Virkkunen et al., 1989; Virkkunen et al., 1989). Fourth, serotonin agonists can reduce aggression in a variety of animal models and in man (Broekkam et al., 1989; McMillen et al., 1988; McMillen et al., 1988).

Obviously, there is considerable overlap in the findings from studies of these different conditions. Perhaps they are all reflective of the same basic phenomenon, that impairment in central serotonin mechanism leads to increased impulsivity which may display itself as outwardly directed aggression in the case of violent offenders or inwardly directed aggression in the case of violent suicide attempters or suicide completers. Conceivably, alcohol causes, in some individuals and/or exacerbates in others, this serotonin impairment increasing the likelihood aggression will occur. If this explanation is the case, knowledge of this central role of serotonin in these forms of psychopathology would be of great value.

SINGLE OR MULTIPLE MECHANISMS

Undoubtedly, the link between central serotonin function and all of these psychiatric disorders is more complex than simply too little or too much. If so, then understanding the role of central serotonin mechanisms would be of even greater value in teasing apart these behavioral syndromes and providing more specific treatments.

For example, the principal reasons for suspecting that dysfunction in central serotonin mechanisms are not all mediated through increased impulsivity are as follows: First, serotonin projects to and innervates brain regions which are not associated with impulsivity or aggression. Second, drugs which have effects on 5-HT2 and 5-HT3 receptors do not have selective effects on aggression or impulsivity despite having other potent clinical actions such as antidepressant and antimigranous effects respectively. Third, the agents having the most selective effects on impulsivity and aggression are agents which are highly selective for 5-HT1a receptor activity such as the azapirones. Even the potency of these agents in terms of reducing aggression can vary depending upon the animal model of aggression being tested (e.g., isolation-induced aggression versus predatory aggression). Fourth, serotonin reuptake inhibitors (SRIs) can have variable effects on aggression ranging from reducing to increasing it depending upon the animal model being used. Consistent with this finding is the fact that serotonin reuptake inhibitors, even though they are selective in terms of the reuptake mechanism, are nonselective in terms of affecting serotonin receptor subtypes since they block serotonin reuptake similarly throughout the brain.

Of note, 5-HT2 receptors downregulate in response to increased serotonin stimulation and upregulate in response to a serotonin deficiency. In contrast, 5-HT1a receptors appear to be more resistant to such effects. This difference might help to explain the differential behavioral pharmacology of serotonin reuptake inhibitors, 5-HT2 antagonists, and 5-HT1a partial agonists.

In major depressive disorder, there appears to be an increase in 5-HT2 receptors in the neocortex (Arango et al., 1990; Arora & Meltzer, 1989; Demeter et al., 1989; Lawrence et al., 1990; Mann et al., 1989; Meyerson et al., 1982; Stanley et al., 1982). In at least some cases, this upregulation appears to be in response to a deficiency of central serotonin. In other forms of depressive disorders, the increase in 5-HT2 receptors may be the primary defect. Treatment with serotonin reuptake inhibitors induces a downregulation of 5-HT2 receptors which coincides temporally with the relief of the depressive disorder. During the same time interval, there is a decrease in the formation and storage of central serotonin and a decrease in the firing of central serotonin neurons. Due to the presence of the reuptake inhibitor and the change in 5-HT2 receptor number and presumably function, serotonin transmission at these sites is presumably more efficient which results in relief of the depressive symptoms.

Not all depressive disorders respond to serotonin reuptake inhibitors. Some respond to 5-HT2 antagonists instead or to the addition of a 5-HT2 antagonist to a reuptake inhibitor. This phenomenon could be explained by a primary defect in the 5-HT2 receptor itself. Such a defect might be relatively resistant to the indirect action of a serotonin reuptake inhibitor. Thus, there is evidence to suggest there may be more than one type of major depressive disorder having a serotonin-based pathophysiology.

At the same time that serotonin reuptake inhibitors are normalizing serotonin function at 5-HT2 sites, they could produce a deficiency of serotonin at the 5-HT1a site. This scenario would result from the decreased firing of serotonin neurons and the resistance of the 5-HT1a receptor to change in response to a change in serotonin tone. As mentioned above, central serotonin stores, CSF 5-HIAA concentration, and the firing rate of serotonin neuron falls after chronic treatment with serotonin reuptake inhibitors. This picture is reminiscent of the state seen in certain animal models of aggression, in violent offenders and violent suicide attempters, and in successful suicides. If the patient being treated already has a relative dysfunction in serotonin function in brain regions rich with 5-HT1a receptors, it could provide an explanation for reports of increased impulsivity and suicide preoccupation in a subset of patients being treated with reuptake inhibitors (Teicher et al., 1990).

IMPLICATIONS FOR PSYCHIATRY

The implications of our evolving knowledge of the brain are substantial for psychiatry and the treatment of patients (Cloninger, 1987; Zuckerman, 1984). The story of serotonin and its receptor subtypes is but one specific example that could also have been made with several other neurotransmitters or neurohumor. The implications include: (a) the recognition that the final clinical effect is the result of the interaction between a given drug's mechanism of action and the patient's physiology, (b) that advances in drug therapy can be used as tools to aid us in understanding the pathophysiology underlying major psychiatric disorders and, as such, changes in psychiatric nomenclature will likely follow developments in psychopharmacology, (c) that such developments in neuroscience may blur the boundary between what is pathology and what is normal function, and (d) that psychiatrists will need to continually refine their skills as behavioral pharmacologists to rationally use the new tools being provided by neuroscience.

From this discussion, a drug's mechanism of action can obviously no longer be viewed as the sole determinant of the clinical effect. Such mechanisms are primarily defined by research using laboratory animals who have simpler and normal functioning mammalian brains. In contrast, these medications are used clinically to treat patients with presumable disturbed brain function. Thus, the clinical effect will be determined by the interaction between the drug's mechanism of action and the disturbance in brain function causing the disorder being treated. Hence, a drug may have substantially different effects in different illnesses, particularly drugs which have relatively broad tonic effects on neurotransmission. The example used above was the effect of serotonin reuptake inhibitors. The leading theory for how these agents treat depression involves the induction of downregulation of 5-HT2 receptors in the neocortex. If the patient's problem is more one of irritability and aggressivity and if that were mediated by reduced tone at 5-HT1a receptors in limbic system structures, then the reuptake inhibitor could theoretically worsen the condition.

Such a drug:pathophysiology interaction could explain apparent paradoxical effects in a subset of patients being treated with a given drug or class of drugs. By being alert to this possibility and by knowing the relevant neuroscience, the clinician could use such observations to better characterize his/her patient's condition and adjust the treatment strategy.

In the same way, researchers can use highly selective drugs as probes to assess the functional integrity of specific transmitter systems in given disorders. For example, the differential effects of reuptake inhibitors versus 5-HT2 antagonists could potentially separate out two forms of depressive disorders: one in which the primary problem is a deficiency in the formation and release of serotonin, and the other in which the primary problem is excessive 5-HT2 function.

While the drugs that are becoming available now are a significant leap forward in terms of specificity of action, they are simply the harbingers of the future. Even more specific agents will become possible given our increasing knowledge of the structure and function of the brain and the increasing sophistication of available tools such as the ability to clone the genes which encode receptor structure. Even now medicinal chemists have a better idea of which new molecular entities to select for drug development based upon continually improving understanding of structure:activity relationships important to psychotropic drug action (Bockaert et al., 1990; Hibert et al., 1990; Seggel et al., 1990).

The development of such agents and the improved understanding of brain function and its relationship to psychopathology is synergistic. These advances are likely to lead to refinements in our psychiatric nomenclature and our understanding of the relationship between normal brain function and psychopathology. With these developments will come a myriad of new issues.

ACKNOWLEDGEMENT

This work was supported in part by the Psychiatric Research Institute at St. Francis Regional Medical Center, Wichita, Kansas. The author thanks Ms. Pamela Veneklasen for manuscript preparation.

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FRAGILE X SYNDROME & FOLIC ACID

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INTRODUCTION

Interest in possible therapeutic effects of folic acid for individuals with fragile X syndrome followed awareness of its importance in the chromosomal culture techniques used to identify the fragile site at the tip of the X chromosome (Sutherland, 1977). Ordinary techniques for preparing chromosomes from blood samples, in order to study them under a microscope, require that the blood cells be bathed for a period of time in a mixture of nourishing chemicals including folic acid. This enables the cells to grow and start dividing, the best conditions for viewing chromosomes. However, in order to show the fragile site, the chromosomes must be cultured in a solution of chemicals which is deficient in folic acid. Often further noxious chemicals are added which stress the developing and dividing chromosomes and make the fragile site more clearly visible.

Folic acid is a naturally occurring essential dietary component. It is necessary for the formation of the walls of red blood cells. We need these to carry oxygen around our body. Any lack of folic acid will result in anemia (not enough red blood cells) and consequent symptoms including breathlessness, weakness and fatigue. Happily, folic acid is present in many common foods, in particular vegetables and fruits including brussel sprouts, spinach, cauliflower and potatoes. Many breakfast cereals such as cornflakes and branflakes are fortified as well. This means that essential nutrients including vitamins and folic acid are added during manufacture to make the food healthier. Folic acid is also present in milk and yeast extracts (e.g., Marmite, Vegemite). Folic acid's importance is demonstrated by its frequent prescription for pregnant women to prevent anemia due to the added nutritional demands.

Initial reports of beneficial effects of folic acid for behavioral disturbances in individuals with fragile X syndrome were published approximately a decade ago (Lejeune, 1982). The logic underlying the therapeutic trial was in fact somewhat flawed. It was proposed that since chromosomes from fragile X individuals needed to be cultured in solutions deficient in folic acid in order to reveal the defect, then maybe individuals with fragile X had a lack of folic acid in their bodies, or were unable to make optimal use of folic acid they had. Therefore, it was argued that supplementing their dietary intake might remediate adverse developmental and behavioral effects of the condition. Early anecdotal reports did indeed demonstrate a gratifying improvement in behavioral function.

There followed confirmation of observed improvement in behavioral function on the basis of subjective parent and teacher ratings (e.g., Rogers & Simensen, 1987). It was also noted that only a proportion of children given folic acid benefited -- other children remained unchanged behaviorally (Carpenter et al., 1983). Also, children seemed to benefit more than adults (Froster-Iskenius et al., 1986). The scientific basis of these claims was enhanced by a well-conducted study which compared active folic acid medication with placebo -- a medicine in every way identical except that it lacked any trace of folic acid (Hagerman et al., 1986). The trial was double-blind -- that is, neither the children, nor their parents or teachers, nor the doctors, knew which of these two medicines were used, and what the order of administration was until the study had finished and all ratings obtained. The researchers found that traditional standardized instruments designed to measure behavioral change were not particularly good in showing improvement while on folic acid. However, the parents and doctors were usually in no doubt as to which medicine was which, even before they were told. The folic acid was much more beneficial. An ensuing literature review (Bregman et al., 1987) described four well-designed studies involving a total of 14 prepubertal subjects and contrasted these with four studies involving 14 post-pubertal subjects. It was concluded that, despite methodological differences in the studies on prepubertal subjects, similar findings were reported including significant diminution in hyperactive behavior with concomitant increase in attentional ability. By and large no changes were noted in either intellectual functioning or language ability. In contrast, results from studies on post-pubertal individuals failed to demonstrate consistent improvement in intellectual function, language ability, activity level or attention span. These conclusions suggested that folic acid may decrease behavioral problems associated with hyperactivity (attention deficit disorders) among pre-pubertal children with fragile X syndrome.

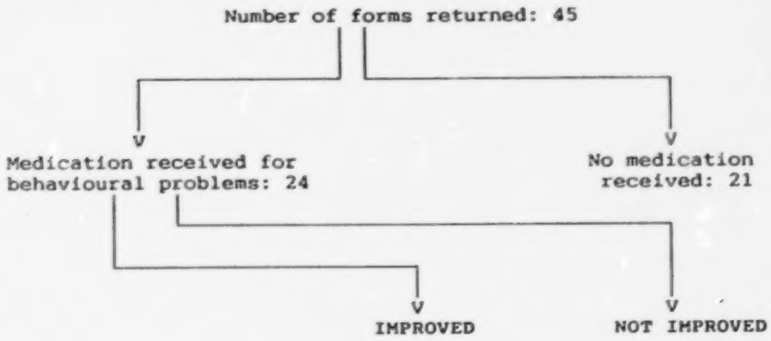
Unfortunately, not all studies have yielded such promising results. Assessment of identical twins using an impressive battery of behavioral instruments failed to demonstrate beneficial effects (Rosenblatt et al., 1985) as did controlled study of three unrelated children (Madison et al., 1986). A further double-blind trial using direct observation and behavioral rating scales also demonstrated no significant improvement on folic acid compared with placebo (Fisch et al., 1988). However, this project did use substantially higher doses of folic acid, raising the possibility that too much medication can be as useless as too little. More recent research has also often failed to confirm improvement in social behavior, intellectual function or attention span, although the persisting importance of a structured, predictable, well-staffed school environment with clear individually defined academic goals and a minimum of distractions and changes in routine has been reconfirmed (Webb et al., 1990).

In view of these conflicting reports, the absence of readily available and widely acceptable medications for hyperactivity in the United Kingdom, and growing numbers of requests to try folic acid, it was decided to undertake further evaluations.

THE BRITISH FRAGILE X SOCIETY MEDICATION SURVEY

A questionnaire was distributed with regular mailings to all members of the Fragile X Society. Enquiries were made as to whether the recipients' children had ever been on medicine for behavioral symptoms. If so, which medications, between what dates, at what dose and how often? Questions were asked such as for what specific behavioral problems the medication was given, whether it was helpful, and if so in what ways. Finally, basic information on the child's gender and age were obtained.

Results are summarized in the table below:


Number of forms returned: 45
Medication received for behavioural problems: 24
No medication received: 21
IMPROVED
NOT IMPROVED

	IMPROVED	NOT IMPROVED
FOLIC ACID (16):	10 (62.5%)	6 (37.5%)
STIMULANTS (4):	1 (25%)	3 (75%)
OTHER (7):	4 (57%)	3 (43%)

Out of 45 forms returned, 24 children had received medication for behavioral problems. Of these 16 had been given folic acid, 4 a more traditional stimulant medication e.g., methylphenidate (Ritalin), and 7 a different drug, e.g., sedatives, tranquilizers, antihistamines. Approximately two-thirds of children who had received folic acid were reported as improved behaviorally. Improvements associated with folic acid were usually related to improved concentration with some evidence of diminished restlessness and defiance. Features associated with non-responsiveness to folic acid included age (having already passed through puberty), low dosage (5 milligrams daily or less) and more deliberate misbehaviors. Features associated with responsiveness to other medications like sedatives included mainly fears and phobias, insomnia and tantrums.

THE INSTITUTE OF CHILD HEALTH/GREAT ORMOND STREET FOLIC ACID STUDY (Giannopoulou et al., 1991)

On the basis of the above encouraging reports it was decided to undertake a scientific evaluation of folic acid for features of hyperactivity and attention deficit disorders in children with fragile X syndrome. It was hypothesized that folic acid is useful in treating overactivity, restlessness, inattentiveness and impulsiveness when found in association with fragile X syndrome. Subjects for the study are children (pre-pubertal and post-pubertal) with fragile X syndrome confirmed on chromosomal analysis, many of whom have already been part of the earlier study defining the characteristic behavioral aspects of children with the condition. Many, but not all, of the candidates for the study demonstrate substantial attentional deficits. None of them are receiving medication that interferes with the body's ability to use folic acid.

Subjects are assigned randomly to two groups. One group receives folic acid medicine for six weeks, followed by placebo medicine for six weeks after a two-week break in between. The other group receives the above medicines in reverse order. Dosage is five milligrams morning and evening. Medicines have been donated by R.P. Drugs Limited to whom we are very grateful for their generosity. Children, parents and clinicians are unaware of the order of medications for each child. Only the pharmacists know, and they are sworn to secrecy until the trial is concluded.

Assessment of the children's behavior is undertaken by a variety of means. Questionnaires are completed regularly by the child's parents and teachers. Interviews with the clinician provides further data on the child's developmental level and behavioral tendencies. Finally, video taping is undertaken. This consists of half an hour during which the child plays with toys initially on his or her own, then with a parent, and finally with the clinician. Records are kept of how much of the time is spent playing with the toys, how much time is spent staying seated at the table, and what proportion of the time is taken up with fidgety behavior. These assessments are undertaken before the study commences and after each course of medication.

In order to test out this format, two boys with fragile X and marked hyperactivity were taken through the protocol. Their ages were 5 years and three months, and eight years. The only difference was that each medication was given for three months rather than six weeks. At the end of this pilot study when the code was broken, both boys had been given placebo medicine first, and then active folic acid.

Parents had no doubt their sons experienced dramatic behavioral improvements while on folic acid. In both cases they were able to guess when folic acid was prescribed. They were sufficiently impressed to request continued prescription of folic acid after the study was finished. In contrast parents were unimpressed with the placebo medicine. One mother described it as the water medicine "because it's as useful and unuseful as giving tap water."

There is some human and experimental evidence that folic acid depletion causes a decrease in brain serotonin (5-hydroxytryptamine) turnover (Botez et al., 1979; Botez et al., 1982). Serotonin is an important neurotransmitter -- a chemical which passes messages between nerve cells in the brain. Folic acid depletion has also been reported as being associated with reduced turnover of dopamine, another important brain neurotransmitter, implying that there may be a correlation between levels of folic acid in the brain and surrounding cerebrospinal fluid, and neurotransmitter production (Singer et al., 1980). It is therefore possible that folate supplementation may increase serotonin turnover, although as yet there is no direct evidence for this. Consistent with this view are findings that the distributions of methyltetrahydrofolate (a metabolite of folic acid) and serotonin in rat brains are identical, supporting a possible role for methyltetrahydrofolate in serotonin metabolism (Korevaar et al., 1973).

CONCLUSIONS

Results of the pilot study and questionnaire survey are consistent with the hypothesis that regular folic acid may be useful in the treatment of restlessness and poor concentration in children with fragile X syndrome. There is now the need for scientific evaluation of this hypothesis using sensitive, standardized behavioral rating instruments on a large number of subjects. This will also allow for investigation of possible effects of age, nature and severity of challenging behaviors, and gender. Such a study is currently in progress.

If results confirm beneficial effects of folic acid in treating hyperactivity in fragile X children, it will be important to evaluate the efficacy of folic acid in children with attention deficit disorders who do not have fragile X syndrome. This is because the way that folic acid seems to be working probably has little, if anything, to do with the presence or absence of fragile X syndrome, but probably a lot more to do with the presence of hyperactivity and attentional problems. A study undertaken in the United States confirmed that children with fragile X and hyperactivity who were given stimulant medicines (methylphenidate) responded in a very similar way to those who were given folic acid (Hagerman et al., 1988). This suggests folic acid, like stimulant medicines, may be acting directly on the brain to enhance the activity of parts which may be slow in developing and therefore not functioning as well as they should. These brain areas would be those concerned with the ability to concentrate and attend to tasks, to avoid extraneous distractions and the tendency towards impulsive thoughts and acts. The effect is analogous to our efforts to help ourselves keep going on relatively uninteresting tasks by drinking mugs of strong coffee or tea. These beverages are packed with stimulants, the best known being caffeine. We take these stimulants to enhance our concentration and resistance to impulsiveness and distractibility.

This proposition still needs to be confirmed by prescribing folic acid for children with hyperactivity who do *not* have fragile X. Also, the issue is raised as to whether some children with fragile X could benefit from more traditional stimulant medications. The answer is certainly yes -- but this needs to be in the context of continuing comprehensive psychological, educational and social interventions.

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PSYCHOSIS IN FRAGILE X SYNDROME

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The issue of psychosis in fragile X syndrome today must proceed along two intertwined paths: diagnosis and treatment of our fragile X patients according to current knowledge, and an assessment of the links between the known molecular genetics, known neurochemistry and the psychiatric symptomatology we have seen in children and adults with fragile X syndrome. The fragile X syndrome has a wide variety of manifestations, from some affected individuals with only uncomplicated mental retardation, to some with retardation, a pervasive developmental disorder (e.g., Autistic Disorder) and psychosis. It is unknown what percentage of fragile X patients are psychotic.

A psychosis is a major psychiatric disorder, the hallmark of which is failure to distinguish between real and unreal. The most dramatic symptoms of a psychosis with disordered thinking are hallucinations (sense perceptions with no basis in the outside world) and delusions (a fixed false belief recognizably bizarre in nature, but susceptible to change by contradictory information). The most dramatic symptoms of a psychosis with disordered mood (often called mood disorder) are intense elation (mania) or depression, each with no obvious cause in the outside world, with actions based upon the mood. Disorganized thinking and disorders of sleep, appetite and social functioning are seen in both types of psychosis. Mixed types of psychoses can and do occur. The most common psychosis with a disorder of thinking is Schizophrenia. The most common psychoses with mood disorders are Bipolar Disorder (once called Manic-Depressive Psychosis) and Major Depression. Mixed types are called Schizoaffective disorders.

Autistic Disorder, commonly called autism, is *not* a psychosis. Once thought to be a very early onset form of schizophrenia, it is now recognized to be a pervasive developmental disorder.

The issue of diagnosis of psychiatric disorders in individuals with mental retardation and pervasive developmental disorders presents several technical problems. Standard diagnostic criteria for currently-recognized psychiatric disorders are set forth in the Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM-III-R) (American Psychiatric Association , 1987) and, in an alternative scheme, in the International Classification of Diseases, Ninth Edition, (ICD-9) (U.S. Department of Health and Human Services, 1989). Both books embody an ongoing review of these criteria as our understanding advances. Neither at this time goes beyond a rudimentary attempt to address the ways in which the various manifestations of psychiatric disorder are modified in an individual with mental retardation or a pervasive developmental disorder such as autism (currently called Autistic Disorder). Neither directly addresses the question of unique or mixed disorders particular to biologically-caused syndromes such as fragile X syndrome. Indeed, the idea is at a crossroads, with some arguing for grouping symptoms meeting diagnostic criteria for various disorders in existing categories, resulting in multiple diagnoses (for example, in the DSM-III-R multiaxial system, an individual with fragile X syndrome might have a diagnosis as follows: *Axis I*: (1) Panic Disorder, (2) Bipolar Disorder, Manic; *Axis II*: (1) Autistic Disorder, (2) Moderate Mental Retardation; *Axis III*: fragile X syndrome). Others would argue for recognition of the various manifestations in such an individual with a diagnosis of *Axis I*: Organic Personality Disorder, *Axis III*: fragile X syndrome.

The first alternative offers the short-run advantage that medical treatment for psychiatric disorders is usually organized around currently-recognized disorders. The second alternative recognizes that in the long-term, as we learn the molecular mechanisms underlying the various psychiatric disorders, we will find molecular links among seemingly different psychiatric problems and perhaps several molecular mechanisms leading to a single common pathway of a single psychiatric disorder. Particular kinds of molecular dysfunction could cause signs and symptoms of meeting diagnostic criteria of two or more currently-recognized psychiatric disorders. Just as pneumonia may be caused by many different organisms, schizophrenia, for example, could be caused by the molecular failure of any number of neurochemical mechanisms. Identification of these mechanisms leads to more appropriate subtyping which would be pertinent for treatment. For example, schizophrenia in an individual with fragile X syndrome might have to be treated with different medication than schizophrenia in an individual with, for example, fetal alcohol syndrome.

The way ahead lies through the investigation of the molecular and neurochemical mechanisms underlying psychiatric symptomatology. I believe the contribution of clinical psychiatry to this effort should be the increasing recognition of the concepts of behavioral and psychiatric phenotype -- those patterns of behavior, and those psychiatric disorders (i.e., patterns of behavior which meet diagnostic criteria for psychiatric disorders) occur with increased frequency in individuals with known biological syndromes. The following sections of this paper will discuss the psychiatric phenotype of fragile X syndrome, touch on possible links between molecular and neurochemical mechanisms underlying the psychiatric phenotype, and propose a rationale for treatment.

THE PSYCHIATRIC PHENOTYPE

Ten years' exposure to affected children and adults with fragile X syndrome have yielded a picture of, in the most severely affected children, severe hyperactivity which in early childhood to early adolescence (and possibly later) may respond favorably to stimulant medications such as Ritalin or to folic acid in the dosage range of 10 to 20 mg/day (Turk, 1992). Some of these children go on to develop a mixed psychosis with, in most patients, a predominant mood disorder presentation, with manic episodes (elation, sleeplessness, severe motor overactivity, irritability, hypersexuality) or even chronic mania, more rarely episodes of depression (severe withdrawal, sleep disorder, episodes of crying) or a mixture of symptoms of mania and depression. In addition, some show evidence of auditory hallucinations and disorganized or bizarre thinking (Hagerman, 1991) . The latter may even be the most prominent symptoms. Under the current diagnostic classification system, depending on the prominence of these various elements, diagnoses would include Bipolar Disorder (Manic) with Psychotic Features, Bipolar Disorder (Mixed) with Psychotic Features, or Schizoaffective Disorder (Bipolar Type).

The standard medical approach to treatment of these disorders would be an antimanic medication (lithium, carbamazepine [Tegretol], valproate [Depakote]) and an antipsychotic medication (any of the many phenothiazines [e.g., Mellaril], thioxanthenes [e.g., Navane], dibenzaepines [e.g., Loxitane], the butyrophenone, Haldol, or the indolone, Moban).

The diabenzazepine clozaril (Clozapine), recently made widely available in the United States, is an antipsychotic which appears to work by a novel mechanism. Clozapine will be discussed further below.

MOLECULAR AND NEUROCHEMICAL MECHANISMS OF PSYCHIATRIC DISORDER

Until recently, the manifestations of psychiatric disorders were attributed to disordered functioning of neurotransmitter systems, the mechanisms by which fairly simple biochemicals such as gamma-aminobutyric acid (GABA), dopamine (DA) and serotonin (5HT), as well as more complex peptides (enkephalins), cross the gaps between neurons to activate or inhibit signal transmission in the next neuron in the chain. Much of what was believed was inferred from known mechanisms of drug action. For example, schizophrenia was widely believed to be caused by overactivity of DA mechanisms in the limbic system of the brain, thought to be the origin of the hallmark hallucinations and delusions. Even this simple view encompassed possible dysfunction of synthesis of DA, release of DA, the DA receptor in the receiving neuron, DA reuptake mechanisms in the transmitting neuron, and DA inactivation mechanisms in the synapse (the gap between the transmitting and receiving neuron). Complexity began to increase when it was recognized that there were several different DA receptors (currently at least five are known).

The hallucinations and delusions of schizophrenia are preceded in most patients by periods of sometimes years of increasing social withdrawal and poor social judgment, cognitive changes, and, after the onset of psychosis, an apparent ongoing dementing process. Recognition of the neurochemical basis for this process as an underactivity of DA in the frontal cortex gave rise to the current theory that schizophrenia begins with falling DA activity ("hypodopaminergia") in the frontal cortex with consequent increase in DA activity ("hyperdopaminergia") in the limbic system (Davis et al., 1991). Alternatively, hyperdopaminergia in the limbic system could cause hypodopaminergia in the frontal cortex by feedback mechanisms (Weinberger et al., 1992).

In either case, the standard treatment of schizophrenia, using drugs which block DA activity, improves the dysfunction in the limbic system, ameliorating or eliminating the hallucinations, delusions, and disorganization of thinking, but either has no effect upon, or perhaps even worsens, the dysfunction in the frontal cortex. The sole exception currently available in the United States is Clozapine, a drug whose mechanism of action is not yet fully understood (Farde et al., 1992; Meltzer & Stockmeier, 1992), but which appears to act favorably upon the limbic hyperdopaminergia while sparing, at least relatively, cortical (cognitive) and basal ganglia function (the latter is the basis for the undesirable movement disorders seen with the standard antipsychotic medications).

Dysfunction of neurotransmitter systems does not satisfactorily explain Bipolar Disorder. The action of antidepressant medications can indeed be explained by their effect on the norepinephrine (NE) and serotonin (5HT) neurotransmitter systems, but these drugs can precipitate mania in susceptible individuals. The antimanic drugs, on the other hand, do not appear to act at this level of neurochemical function. Lithium appears, rather, to act upon what has come to be called the second messenger system (Baraban et al., 1989; Manji, 1992). Finally, antipsychotic medications favorably affect acute mania.

The first messenger to affect the receptor neuron is the neurotransmitter. In a few cases, older theory holds true that coupling of the neurotransmitter with its receptor site (a protein at the cell membrane) causes ion flow across the membrane and propagation of a nerve impulse in the receptor cell. It is now known that the more common response of the coupling of the neurotransmitter to its receptor triggers a complex cascade of chemical reactions *within* the receptor neuron. These reactions can separately or simultaneously lead to propagation of a nerve impulse or inhibition of a nerve impulse. These reactions also interact with the chemical cascades triggered by *other* neurotransmitter/receptor sites in the same cell (resulting in summation of inputs from various neurotransmitters leading to propagation or inhibition of a nerve impulse: a chemical "transducer" mechanism). In addition, the synthesis or inhibition of synthesis of neurotransmitter molecules or transducer proteins (by turning on or off chromosome-based synthesis activity) is also influenced by second messengers.

The links between the molecular mechanisms now thought to underlie events at the fragile site at Xq27.3 and the neurochemical events underlying the psychosis we sometimes observe in the most severely affected individuals are as yet unknown. There are, however, several interesting and suggestive observations. Any inferences drawn from these observations are obviously highly speculative, but they provide a rationale for clinical trials of new approaches in a situation in which, alas, standard approaches to treatment have yielded at best limited success.

First, the second messenger system's mechanisms provide a new basis for understanding both cognitive functioning and psychiatric disorder. The second messenger system as it is currently understood relies upon the chemical activation of a series of molecules. Each reaction in the series is controlled by a protein coded somewhere in the genome. Neurotransmitter receptors are also proteins whose makeup is coded in the genome, and whose rate of synthesis is controlled by other proteins coded in the genome. The recent cloning of the FMR-1 gene at Xq27.3, and investigation of the effects of the CGG repeat (Fu et al., 1991; Rousseau et al., 1991) have suggested that the product protein is likely to be a regulator of the synthesis of other proteins, and that the effect of higher numbers of CGG repeats is to cause the "turning off" of the gene in affected individuals. It is easy to see how the absence of a protein crucial to regulation of one or several components of the second messenger's transduction system could cause instability within the system, with resulting dyscontrol and perhaps oscillating functioning of some or many crucial neuronal networks.

The impression that such a mechanism could explain the oscillating, uncontrolled mood swings of Bipolar Disorder is strengthened by the discovery that the antimanic drug lithium does decrease the activity of an apparently overactive protein in the second messenger cascade.

The same result could occur by indirect genetic mechanisms as yet unknown. It appears that the location of a gene, its proximity to other genes on the same chromosome, can be crucial. There is, as mentioned, dispute over the exact action of Clozapine. The earliest explanations were based on the drug's relative affinity for D1 and D2 (dopamine) receptors. More recently, the D4 receptor has been implicated, due to Clozapine's apparent high affinity for D4 receptor binding (about ten times its affinity for D2 and D3 receptors). The D4 receptor exists in at least three polymorphic variants (Van Tol et al., 1992), the variant alleles being produced by repeats in the coding sequence similar to those seen in the FMR-1 gene. The location of the D4 receptor gene at (chromosome) 11p15.5 is just distal to the Harvey *ras*-1 locus (Gelernter et al., 1992), a region that has been implicated in familial Bipolar Disorder (Kelsoe et al., 1989), a disorder which responds at least partially to Clozapine. Also, receptor proteins react directly with second messenger system proteins. A cursory examination of a map of the distal end of the long arm of the X chromosome quickly discloses the proximity of the FMR-1 locus to loci linked to synthesis of HPRT (the enzyme deficient in Lesch-Nyhan syndrome, a mental retardation syndrome characterized by severe hand, arm and lip biting), GABA-alpha3, a receptor protein for the neurotransmitter GABA (implicated in Panic Disorder), and the SF14 locus associated the Familial X-linked Bipolar Disorder by Medelewicz. Hand-biting, Panic Disorder and mania are all seen in severe fragile X syndrome.

These observations suggest that these proximities are not coincidental, and that as yet unknown mechanisms can cause dysfunction at one genetic locus to affect the function of adjacent loci. Since not all affected individuals with fragile X syndrome have a psychosis, it is possible that the repeats at FMR-1 are not the only necessary condition for the occurrence of mood disorder. Elucidation of the putative link between nearby chromosomal loci may provide the answer. The link between FMR-1 dysfunction and mood disorder, then, could be direct, via direct effects of the absence of FMR-1 protein; indirect, via effects on the function of the adjacent locus, both, or by other, as yet unknown mechanisms. A possible final common pathway seems likely to be the second messenger system.

The link to thought disorder is more speculative still. Hypodopaminergia in cortical systems appears to be one mechanism explaining hyperactivity and the therapeutic effects of Ritain and folic acid. Failure of these drugs to effect hyperactivity after the early teen years in patients with fragile X syndrome follows the IQ trajectory seen in the syndrome (Hodapp et al., 1990), and the trajectory of adaptive skills (Dykens, 1992). There is a known physiologic decline of dopaminergic function from birth to late adolescence in normal individuals. Perhaps in fragile X this is more pronounced with a greater imbalance between cortical hypodopaminergia and limbic system hyperdopaminergia with resulting thought disorder in some patients. Since children with Attention Deficit Hyperactivity Disorder with causes other than fragile X syndrome do not go on to develop schizophrenia or other thought disorder at rates higher than the general population, one would have to assume here processes intrinsic to fragile X syndrome that go beyond the normal physiological decline in dopaminergic activity. Two papers presented at this conference are of interest in this regard: Reiss' presentation of preliminary MRI data showing apparent cortical tissue loss in individuals with fragile X syndrome and Berry-Kravis' finding of lowered cyclic AMP (cAMP) synthesis activity in fragile X-affected tissues.

Lowered cAMP activity is known to be associated with decreased learning efficiency in simple organisms. However, cAMP is a compound crucial to the functioning of several different biochemical cascades in the second messenger system, making it a chemical "junction point" between transducer systems integrating second messenger cascades activated by different "first messenger" neurotransmitter systems. In one interesting link, cAMP activity governs the activity of the enzyme tyrosine hydroxylase, crucial to the production of DA. Since cAMP is also crucial to the second messenger cascade known to be involved in mood disorders, there is at least some reason to think that there can be links between the underlying mechanisms of mood disorders and thought disorders, and that novel biological syndromes like fragile X syndrome could produce particular and peculiar mixes of symptoms of the two principal types of psychoses.

At this point, there is far too little hard information to do more than speculate about the biochemistry of psychosis in fragile X syndrome. However, we need to speculate, based upon the latest information, in an effort to optimize existing treatments.

IMPLICATIONS FOR TREATMENT OF PSYCHOSIS IN FRAGILE X SYNDROME

We are left with several lines of speculation regarding the mixed psychosis of fragile X syndrome. First, it is possible the causes of the mood disorder symptoms and thought disorder symptoms proceed along different lines and converge and interact via "cross-talk" between the underlying second messenger systems. In this hypothesis, the inactive FMR-1 gene, directly or indirectly, causes a degenerative syndrome in the cerebral cortex resulting in cognitive processing deficits and hyperactivity. In some individuals, decrease of cortical DA activity triggers increased DA activity in the limbic system, giving rise to hallucinations and delusional and disorganized thinking. Simultaneously, either by direct or indirect effect of the inactivity of the FMR-1 gene, or by its effect on the nearby St14 locus, mechanisms are set in train to produce the dysfunctional second messenger cascade underlying mood disorder. "Cross-talk" between the two systems affects the mix of symptoms displayed by any one patient. A variant of this hypothesis would have some deeper-lying mechanism causing both cortical hypodopaminergia and limbic hyperdopaminergia.

A second hypothesis would postulate a direct connection between the inactive FMR-1 gene and the second messenger systems underlying mood disorder and limbic dopaminergic overactivity. Both hypotheses could account for great variety in symptom presentation. There is far too little information available at this point to rule in or out either of these hypotheses. In the meantime, we must try to tailor treatment of the psychosis with most prudent attention to the possibilities. The first hypothesis would suggest early and aggressive treatment of hyperactivity with stimulant medication. It must be remembered that only the hyperactivity is certain to respond to such treatment -- this hypothesis says nothing about effects on aspects of retardation or pervasive developmental disorder. We hope not only to ameliorate hyperactivity (a worthy goal in itself, of course), but to delay, ameliorate, or prevent the onset of symptoms of thought disorder.

The second implication of the hypothesis, that psychosis is in part a result of or associated with cortical hypodopaminergia, is that when thought disorder symptoms appear they should be treated with an antipsychotic that spares to the greatest extent possible cortical dopaminergic activity. Clozapine is the first of a new generation of antipsychotics which does this. However, there have been no studies of the use of Clozapine in fragile X. In addition, compared to other antipsychotics, Clozapine has a far greater risk of causing leukopenia which, if undetected, can proceed to marrow suppression. For this reason, white blood cell counts must be followed weekly and documented before the weekly dose is dispensed. Also for this reason, Clozapine cannot be used together with other drugs which lower white cell count, most prominently carbamazepine (Tegretol). Given the frequency of seizure disorder in fragile X syndrome, this is no small problem. For the same reasons, when thought disorder symptoms occur together with mania, lithium is the antimanic drug of choice since there is a high probability that Clozapine will have to be used at some point.

The third implication of the hypothesis is that, pending further illumination of the observations suggesting degenerative processes, we should, where possible, look to the use of drugs that enhance existing neurotransmitter activity (for example, "re-uptake blockers") while awaiting the development of interventions that directly address underlying biochemical dysfunction.

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II. Educational & Behavioral Interventions

EDUCATIONAL STRATEGIES FOR CHILDREN WITH FRAGILE X

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Recently, much has been written about the cognitive and behavioral phenotype of children with fragile X syndrome (fra X) (Hagerman & Silverman, 1991; Goldson & Hagerman, 1992). Awareness of characteristics often associated with fra X may help teachers and parents identify the physical, social and learning styles, strengths and needs of children with fra X. However, those of us who live and/or work with children who have fra X are aware of the diversity of their individual strengths and needs. Because there is so much variability in the social and educational needs of children with fra X a single curriculum or standard course of therapy is not advised. Instead, careful development of an individualized education program (IEP) based on the specific strengths and deficits of each child is required. The purpose of this paper is to provide a brief overview of strategies that may be useful for addressing the social and educational needs of children with fra X.

EARLY INTERVENTION

Researchers, parents and teachers have long felt that early intervention results in maximum social and educational benefit for children with developmental delays. Recently, Congress passed legislation (PL 99-457) which mandates early intervention services for children with developmental delays age 3-5 years and some states have extended this mandate to include children from birth to three years.

Language delays are almost always experienced by children with fra X. Speech therapy should be a component of early intervention programs for children with fra X. Suggestions for speech therapy strategies will be provided later in this paper. Most children with fra X are hypotonic and experience fine and gross motor delays. To address these issues, standard occupational and physical therapy strategies can be implemented in early intervention programs. Increasingly, parents, teachers and therapists have begun to identify sensory integration (SI) deficits as a target for early intervention programs. Sensory integration techniques will be described in more detail later in this paper.

In addition to enhancing the child's development and identifying strengths, early intervention professionals can help parents understand the child's developmental delays and challenging behaviors. Early intervention professionals can teach parents to use strategies such as structuring the environment, preventing overstimulation, using therapeutic calming techniques and various positive reinforcement-based techniques.

Participation in early intervention programs that include normally developing children is recommended. Physical, intellectual and social abilities of children with and without disabilities generally are least divergent at young ages and tend to become more pronounced as they grow older. Establishing relationships among children with and without disabilities *and* between their parents may be easier if it occurs earlier versus later in life. Establishment of friendships and expectations that a particular child and his family "belong" to the "group" will help to insure the child has opportunities to participate in a full range of school and extra-curricular experiences during his or her school years.

Participation in integrated preschool and school programs may be particularly beneficial for children with fra X. Incidental learning may occur when children with fra X spend time interacting with non-delayed peers because children with fra X tend to be highly social and often possess strengths in imitation.

PROVIDE STRUCTURE

One strength children with fra X often exhibit is the ability to learn *routines*. Many children with fra X develop nearly "ritualistic" patterns of behavior associated with their daily routines. This occurs as a result of repeating a particular routine over and over (e.g., insistence on dressing in a certain fashion, repeating a particular phrase whenever asked to complete a task, demanding to sit in a specific seat at the table, etc.). It is recommended that teachers and parents should attempt to understand the *function* of such behavior patterns rather than to view this type of behavior as inappropriate and focus on eliminating it. It is reasonable to assume that a child who frequently engages in routines is doing so, at least in part, to provide structure to an otherwise somewhat confusing environment. If the function of routines is to empower the child with self-control by providing enough structure in the environment to allow the child to "know what's coming next" then engaging in a routine may, in fact, be quite adaptive for the child.

SCHEDULE AND MESSAGE BOARDS

It has been established that children with fra X typically have strengths in visual memory and tend to process information better if presented with the whole, or an overall gestalt. Conversely, children with fra X generally do not perform optimally when information is presented only auditorally and have difficulty processing information presented sequentially (Pennington et al., 1992).

Some children with fra X may engage in disruptive or excess behavior during times of transition or when routines are disrupted because they are overwhelmed and/or overstimulated by a world that seems unpredictable. When children do not determine their own activities/schedule they may be confused by transitions and changes in daily routines. Excess behavior that occurs under these conditions may be decreased by providing a means for the child to plan and follow his/her own schedule.

The use of a *pictorial schedule* may allow the teacher and/or parent to capitalize on the child's natural tendency to make a routine of his daily activities while providing information in the medium that he uses most proficiently. The use of a pictorial schedule allows both a whole and sequential look at the days activities for the child and provides a concrete means for teachers and parents to discuss changes in the routine with the child.

Another variation on picture schedule boards is the use of a *message board*. A message board may be used to inform the child of a specific activity or task that needs to be performed. The message board only contains one message at a time. Therefore, it may be less confusing for a child who is just learning to use such a device to provide structure to his day.

INCLUSIVE SCHOOLS

The purpose of schools is to prepare children to function in society as adults. To achieve this goal, all children must feel they belong to the same society. This, in turn, requires children be educated together regardless of the presence or absence of a disability. In addition to focusing on academic skills, children should be given opportunities to master social and interpersonal skills as well as functional life skills prior to graduation from high school. Children with or without disabilities cannot learn to interact and value one another if they do not spend time together. Occasionally, it may be necessary to provide therapy in an individualized fashion. However, every effort should be made to incorporate therapy within settings and activities that occur in the "mainstream" of the school.

CLASSROOM MODIFICATIONS

Mere physical integration is not sufficient for optimal learning for children with fra X. In fact, due to the tendency to become overstimulated easily, many children with fra X may experience difficulties coping with the often stimulating environments of many regular education classrooms. In order to increase the benefit children with fra X receive from participation in general education settings, it is important to provide modifications to the classroom in the following areas: (a) environmental, (b) instructional format, and (c) curriculum content.

Environmental modifications may include modified seats. Seats should be designed to meet the individual needs of the child. The chair back should provide slight pressure at the base of the spine and the seat bottom should be set at a height so that the child's feet are flat on the floor. Furring strips may be attached to the chair to define the outline of where the child should sit. Dycem may be applied to the chair to make the surface non-slippery. Other innovations in seating include using therapy balls, rocking chairs or bean bag style seats. It is also important to consider the location of the seat. The chair should be placed out of the main flow of the room with considerations of hallway and outside noise and distractions. Natural or "full spectrum" light fixtures are recommended as opposed to fluorescent. Be aware of odors from perfume, body and food. Try to minimize exposure to odors. Make an effort to designate a "quiet area" somewhere in or near the room. This place should be made freely available to the child and should not be used as a negative consequence (i.e., time out area). Rather, the child should be encouraged to go to the area to calm down or relax on an "as needed" basis.

The instructional format of any classroom may be designed in one of three ways: (a) in a competitive fashion, (b) individualized and self-paced learning, or (c) learning in pairs or small groups with members helping one another master the assigned material (Johnson & Johnson, 1986). To promote integration into the mainstream of the class, teachers can assign small groups and instruct students to learn the assigned material and to make sure the other members of the group also master the assignment. Learning is assessed frequently using a criteria-referenced evaluation system to ensure all students learn. This type of instructional format has been labelled *cooperative learning* (Johnson & Johnson, 1983).

Curricular modifications will be required in most subject areas for children with fra X. Curriculum modifications must be made on an individual basis. Therefore, specific recommendations cannot be made in this paper. However, in general, teachers should consider the activity, materials and learning objective. Teachers should attempt to modify as few of these learning components as necessary to allow the child to participate in the educational activity. Modifying the activity may involve reducing the complexity of the task or designing a parallel activity. Modifying materials may include highlighting or enlarging text and using manipulative or prosthetic devices. Learning objectives may be modified to include a focus on participation versus mastery.

SPEECH THERAPY

In general, it is important to incorporate therapy techniques and strategies for facilitating communication into the daily activities of children as opposed to providing *only* individual therapy. This may mean that other children, educators and family members will need to be taught to interact with the child using specific speech/communication strategies. For example, if the child is using a communication board and/or sign language it will be important for significant others in the child's life to be taught to use the system (see McClennen & Schopmeyer, these proceedings). This point helps to illustrate the fact that our efforts as educators and family members should not always be exclusively on remediation of deficits of the child with fra X. Often it may be important to teach *other people* (e.g., peers, family members, school staff, members of the community, etc.) the skills necessary to effectively communicate with the child. Likewise, it may be more effective to create and/or modify environments that are more supportive of the particular strengths and needs of the child than to always focus on teaching the child to "fit in" to the setting as it exists. This may require us to reduce the distractions, modify the schedule or sequence of activities and/or change our expectations for performance within a particular place.

Some general suggestions for facilitating speech and communication by children with fra X follow. Many children with fra X have difficulty *initiating* conversation or *making requests*. When speech formulation is difficult for the child or perseveration occurs, the teacher or parent should model desired responses by using parallel talk (i.e., talk about what the child is doing or thinking about) and acknowledge his/her communication *efforts*. Once the child begins to make communicative responses it is important to attempt to get him/her to *expand* his communication responses. Again, the use of parallel talk and *minimal cues* may help the child to expand his communicative responding. It is very important that the educator or parent does not overwhelm the child by talking continuously to fill in conversational voids. The child must be given ample opportunities to use his/her skills. Other suggestions for strategies to address specific issues that may interfere with the child's language/communication development are provided in Table 1 (Adapted from Scharfenaker et al., 1991).

Table 1
Speech Language Deficits and Intervention Strategies

Deficit areas	**Intervention strategies**
1. Attention	- Utilize child's interest areas. - Supplement auditory with visual input including photographs. - Keep auditory and visual distractions at a minimum. - Assign seats away from hall or street noise and not more than 10 feet from the teacher. Such seating allows the child to better utilize hearing and visual cues. - Always gain the child's attention before giving directions or initiating class instruction. Calling the child by name or a gentle touch will serve to alert the child and to focus attention upon the classroom activity. - Ask questions related to the subject under discussion to make certain that the child is following and understanding the discussion. - Use brief instructions. Keep instructions relatively short.
2. Participation	- Have the child read ahead on a subject to be discussed in class so he/she is familiar with new vocabulary and concepts, and thus can more easily follow and participate in classroom discussion. Such pre-tutoring is an important activity that the parents can undertake. - List key vocabulary. Before discussing new material, give the child and parents a list of key vocabulary. In class, put key vocabulary on the blackboard. Try to build the discussion around this key vocabulary. - Visual aids. Visual aids help children with fra X develop skills by capitalizing upon strengths in visual processing and thus providing the auditory/visual association often necessary for learning new concepts and language. - Write instructions. Children with fra X may not follow verbal

	instructions accurately. Help them by writing assignments on the board so they can copy them in a notebook. Also, use a "buddy system" by giving a classmate the responsibility for making certain the child is aware of the assignments made during the day. - Encourage participation in expressive language activities such as reading, conversation, story telling and creative dramatics. - Remember that children with fra X may become fatigued more readily than other children. They may not attend because of the continuous strain resulting from efforts to keep up with classroom activities. Therefore, provide short intensive periods of instruction with breaks during which the child can move around. - Inform parents. Provide the parents with consistent input so that they understand the child's successes and difficulties, as well as the need for individual tutoring at home.
3. Delayed onset of expressive speech	- Utilize a total communication approach-fade out signs as verbalizations increase. - Reinforce any attempts at speech, shape responses by modelling correct utterances.
4. Rate, rhythm, "cluttering"	- Model correct rate/rhythm. - Melodic intonation therapy (Sparks et al., 1984; Sparks & Holland, 1976) - Increase self-monitoring. - Voice synthesizer for auditory and visual feedback.
5. Oral and verbal dyspraxia	- Music, singing, movement - Integrate therapy with occupational therapist (Windeck & Laurel, 1989).
6. Memory	- Elicit attention prior to task. - Pair auditory information with visual cue. - Rhythm and music to cue recall. - Use short instructions intrinsic to task.
7. Abstract reasoning, problem solving skills	- Utilize realistic, meaningful materials of interest to the child. - Begin at level of concrete and systematically increase level of abstraction. - Use of microcomputers/game format. - Think Aloud Program (Camp & Bush, 1981)

8. Verbal perseveration tangential comments topic maintenance	- Allow increased processing time. - Model desired utterances. - Reduce complexity of utterances to child's level. - Monitor anxiety level and adapt accordingly (calming and focusing activities). - Provide opportunities to practice a variety of speech acts. - Redirect the child verbally. - Have the child reauditorize to help process. - Emphasize "topic" through use of high interest materials.

OCCUPATIONAL THERAPY

It is beyond the scope of this paper to describe in detail specific occupational therapy (OT) techniques. Assessment by a licensed OT is required to identify possible OT needs of each child. Likewise, because therapy needs are highly individualistic, it is suggested that parents and teachers seek recommendations from an OT. Involvement of an OT in the child's IEP development can insure that strategies for dealing with issues are incorporated throughout the child's school and home activities.

It is important for parents and teachers to have a general understanding of how some of the manifestations of fra X impact on affected children. For example, it is documented that children with fra X almost always present with loose connective tissue (Hagerman, 1991) and cerebellar dysfunction (Reiss et al., 1988). For children with fra X, the ability to complete fine motor or "paper and pencil" tasks is influenced by decreased muscle strength related to connective tissue disorders as well as impaired coordination resulting from cerebellar dysfunction. Therapeutic interventions are helpful for ameliorating these difficulties. In addition, to increase motivation to participate and lessen the degree of frustration experienced by the child, parents and teachers should select and/or modify activities to reflect her/his endurance, fine motor, balance, and coordination abilities.

SENSORY INTEGRATION

Sensory integration (SI) is defined by the American Occupational Therapy Association as the nervous system's process of assimilating and organizing sensory information for functional use. An individual's ability to interact with the environment is influenced by how effectively and efficiently that person is able to process and use information from the tactile, vestibular, proprioceptive, vestibular, auditory, olfactory, and gustatory systems.

Meeting the SI needs of a child with fra X requires the direct involvement of a professional trained in SI therapy. Many children with fra X experience a number of difficulties which may be amenable to SI therapy including: (a) low muscle tone, (b) delayed gross and fine motor development, (c) poor motor planning skills, (d) toe walking, (e) fear of movement, (f) poor balance, (g) aversion or extreme sensitivity to touch, sound, or visual stimulation, (h) resistance to change, and (i) problems with transitions.

Sensory integration therapy assumes the root cause of these difficulties can be traced to a failure of the nervous system to integrate sensory input properly. This causes hypersensitivity to stimulation which is upsetting to the child and leads to behavior that may appear inappropriate to observers but actually serves an adaptive purpose for the child. For example, from an SI perspective, a child that talks continuously, hand flaps, hand bites, avoids eye contact, or withdraws (all common behavioral characteristics of children with fra X) may be doing so in order to gain a sense of control over what seems like a chaotic and overwhelming environment. These behaviors may function to temporarily block out or mask the overstimulation from a given environment for the child.

The problem of screening out important from unimportant stimuli in the environment experienced by children with fra X may make it difficult to focus attention or relate in other than a stereotypical way to another person. This difficulty may lead to behavior that appears impulsive, hyperactive, disruptive, or aggressive.

A state-by-state directory of SI therapists listing the name, address and phone number of certified SI professionals can be obtained by writing to:

American Occupational Therapy Association
SI Special Interest Section
P.O. Box 1725
Rockville, MD 20850-4375
(Phone: 301 948-9626)

(For further information about SI therapy, the interested reader is referred to Scharfenaker et al., 1991).

BEHAVIOR MANAGEMENT

A number of positive reinforcement techniques can be used to help children learn to control their own behavior (i.e., to self-regulate). Just as a picture schedule and message board offer children a means for structuring their lives, *self-regulation* training has been used to teach children to control their own behavior. Important considerations when using behavior management procedures include: (a) do *not* make the teacher or parent the "source" of reinforcement (i.e., teach the child to deliver his own reinforcement), (b) keep the focus of intervention *positive* (i.e., even if the concern is that a particular behavior is happening excessively, the target response should be an alternative or incompatible response rather than "punishing" the occurrence of the undesired behavior), (c) help the *child set goals* rather than prescribing goals to him, (d) goals should be set and changed *incrementally* (i.e., rather than setting the ultimate goal as the criterion for reinforcement, initially set a goal that is realistically attainable by the child within a week and modify the criterion frequently as the goal is met until the ultimate objective is achieved), and (e) be sure to include at least one skill/activity that the child performs well as a target for self-regulation (to increase the frequency of reinforcement and thereby the child's motivation to use the self-regulation procedure). There are four components of behavioral self-regulation including: (a) *goal setting*, (b) *self-monitoring*, (c) *self-reinforcement*, (d) *adjusting goals*.

The teacher and/or parents may need to take a primary role in helping the child *set goals*. However, it is important the child participates in the process so that she/he feels some ownership, control and responsibility toward achieving the goal. Setting goals requires the target behavior is first operationally defined. This means any two people could view the target behavior and agree on its occurrence. It is particularly important for the child to be able to identify the occurrence of the target behavior(s) since he/she is learning to *self-regulate*.

Teaching *self-monitoring* may require the teacher or parent to devote some instructional time to teaching the child to identify occurrences of the target behavior. To illustrate how self-monitoring can be useful, consider the common problem of topic repetition. During prescribed time periods (e.g., show and tell, group reading and discussion times, and meals at home) the teacher, parent and peers can be taught to help the student learn to self-monitor each time he talks about a new topic. Initially, significant others should point out the occurrence of new topics by saying for example, "Thanks for telling us about the car you saw this morning! That was interesting! Why don't you make a mark on your chart" (push a button on your recording device, drop a bean in your jar, etc.). Notice that although the goal is to reduce topic repetition, the focus of training and reinforcement is *not* to decrease repetition but to *increase* topic diversity. This is an example of keeping the focus of intervention *positive*. Over time, teachers, parents or peers can begin to say, "Hey, what did you just tell us? Did you make a mark on your chart (push the button on your recording device, drop a bean in your jar, etc.) for talking about a new topic?" Eventually, this external labelling of the target behavior should be *faded* to an intermittent schedule.

Although we are only targeting positive goals, it will be necessary for teachers and/or parents to monitor the occurrence of undesirable behaviors. As the frequency of desired behaviors increases the frequency of undesired behavior should decrease. If this does not occur, it may be necessary to change or add another target behavior that is incompatible with the occurrence of the undesired behavior. For example, if the student frequently becomes excited when greeting his peers and subsequently pushes others, it may not be possible to "catch him not pushing" enough to begin to teach "not pushing." One strategy for this situation is to reinforce an incompatible response such as putting hands in pockets while greeting the other child, shaking hands and greeting, or clapping hands and greeting. When choosing an alternative response one must consider what is age appropriate and socially acceptable. Remember the point of teaching an alternative response is to provide an opportunity to teach self-monitoring of the target response (in this case, greeting peers without pushing them). As the child begins to learn the alternative response you should de-emphasize the importance of the alternative and focus on the original target response.

To achieve self-regulation it is critical the child be taught to deliver his own *reinforcement*. If the teacher or parent is always the agent that delivers reinforcement, the child will learn to be dependent on others for rewards. This pattern of behavior may result in behavior that is controlled by the presence of a particular individual (e.g., mom, dad, teacher, grandma, etc.) rather than the child's own volition. Therefore, be sure to set up a contingency with the child that allows him to self-reinforce based on his self-monitoring. Many children will need to be reminded they have "earned" reinforcement initially. To assist the child to learn self-reinforcement use a visual system for self-monitoring (e.g., checks on a chart, tangibles such as coins, slips of colored paper, etc.). Be sure the rules are clear and consistent. Finally, be certain the reinforcer(s) really is desirable to the child.

Teaching children to *adjust their goals* requires they have many opportunities to view the self-monitoring data they collect in relation to the goals they have set. Functionally, this means teachers and parents must adopt self-regulation as a major focus of instruction in order to insure these opportunities exist. It is recommended that daily and weekly, teachers and parents spend time with the child encouraging him to reflect on his performance of the target behavior for each goal. This does not necessarily entail a large commitment in time. It can be worked into the day in a natural way. For example, after dinner parents can ask the child, "How did you do today at school talking about different topics? Show me your bean jar and let's count how many different things you talked about." After counting the beans together (or marks on a chart, etc.) the parents could ask, "How do you think you did? What do you think you will talk about tomorrow at school?" Parents can help the child plan and rehearse behavior for the next school day. Likewise, school staff can help children plan and practice home behavior. To work effectively, parents and teachers must communicate on a daily basis. Many parents and teachers write notes back and forth in a notebook carried by the student. Phone calls also help to clarify important points.

Teaching self-regulation requires the child, teacher and parents to learn a set of component skills just as teaching a child to dress does. We believe the skills involved in self-regulation are as important as any other skill that can be taught in school. In fact, once learned, self-regulation skills can be generalized to nearly every aspect of the child's life. Therefore, it is recommended that IEP's include educational goals and objectives specifying mastery of self-regulation and component skills (i.e., goal setting, self-monitoring, self-reinforcement and adjusting goals).

COMPUTERS

Computer technology holds great promise for children with fra X in the areas of education, communication, leisure/recreation and potentially, independent living. Because many children with fra X have strengths in visual memory and difficulties with auditory processing, computers may be particularly effective for meeting their learning needs. A description of some hardware and educational software can be found in Hagerman & Silverman (1991).

Educational software is now available which may help to make learning of basic concepts (language and mathematics) fun for children with fra X. Computer software may capitalize on the visual memory strengths of learners with fra X while incorporating high interest themes. Computer proficiency may provide children with fra X chances to interact with nondisabled individuals via educational programs, electronic games and vocational opportunities.

In addition to capitalizing on the visual memory abilities of children with fra X, the computer may also serve as a useful alternative to printing or cursive writing. Using a keyboard may help the child overcome difficulties in fine motor and hand strength which can make manual writing difficult. It is important to view the computer as an instrument to *augment* other instructional strategies and capitalize on the motivational and normalizing potential it offers. Parents and teachers are encouraged to *not* "push" computers and/or keyboards on young children; rather, they should let the child explore and have fun using software that is of high interest to the child. Finally, because computers are increasingly becoming part of daily life for us all, one could argue that in the future, computer use may one day be considered a "basic life skill."

ACADEMICS

Many of the topics described above have application to academic instruction. Although there is copious literature regarding special education techniques, a few suggestions have proven helpful for children with fra X and are briefly mentioned below. For more detailed suggestions, the interested reader is referred to the papers by Miezejeski & Hinton and Braden in these conference proceedings.

As mentioned earlier, curricula should include topics and/or materials of high interest to the child (e.g., hobbies, famous people and places, TV and movie characters, etc.). Using high interest materials and themes in the curricula will help to motivate the child to learn and improve his attention. Of course, this strategy applies to all learners, but may be particularly important for children with fra X.

Suggestions for reading decoding instruction include an emphasis on visual associations versus a sequential phonetic approach. We know from analysis of psychological testing data that children with fra X do not do well on sequential tasks (Kemper et al., 1988). Additionally, anecdotal reports from teachers and parents indicate that using only a phonetic approach to teaching reading decoding may not be beneficial for children with fra X. Flash cards highlighting the shape of a word with a picture or symbol representing the word embedded within the word may be helpful for teaching initial vocabulary. As the child begins to master particular words the picture or symbol can be faded from the word until only the word remains. Marcia Braden has developed another technique for teaching reading of words representing high interest topics. Her technique, called *Logo Reading*, is described in more detail in her report in these conference proceedings. Finally, there are a number of educational software programs which teach reading by requiring the learner to match visual stimuli to words.

Reading comprehension may be improved by pairing visual images with words to provide a richer context for the child to interpret their meaning. Perhaps the best example of this can be seen in a book by Marta Campbell (1991) titled, *Say it with pictures*, which can be obtained from the National Fragile X Foundation. Combining line drawings and/or symbols such as Rebus symbols may help the child comprehend the meaning of written and vocal language. Layering communicative stimuli (e.g., vocal, written, line drawing, Rebus symbol, etc.) allows the learner to look at each layer for additional information if he can not comprehend the meaning from a single mode of communication.

Suggestions for teaching math operations again include incorporating high interest topics for the child in the curriculum. Additionally, using manipulatives and "real life" activities may help to make math more comprehensible to children with fra X who generally have deficits in abstract reasoning. For example, cooking simple recipes typically provides a high interest activity with opportunities for teaching math operations using manipulatives (e.g., If you add two cups of water and one cup of flour how many cups will you have?; How many eggs are in the carton?; If you take two eggs from the carton how many are left?). Building simple models and craft projects also affords opportunities to practice math operations. Devising "scavenger hunts" or drawing maps of school, home and community locations may also provide a context in which to stage math problems with intrinsic meaning to children with fra X. Board games may provide a means for teaching math concepts (i.e, counting numbers on dice, identifying numbers on a spinner, moving game pieces forward and backward, etc.).

Finally, it is worth mentioning that some mechanical devices may be appropriate for children with fra X, particularly as they grow older. Most notably is the hand-held calculator. Many children with fra X will not master abstractions inherent in mathematics. However, using a calculator may circumvent this need in much the same way that velcro fasteners on shoes has eliminated the need to teach *all* children to tie their shoes. Using devices or alternative strategies allows the child to functionally do what is required when mastery of the operations necessary to complete the task may be beyond the child's ability. Parents and teachers should carefully consider the "costs and benefits" of prosthetic devices in relation to their child's age, abilities and interests. In general, devices (e.g., calculator) or alternative response strategies (e.g., "next dollar" technique of paying for items by always giving the clerk one more dollar than the item costs rather than trying to count change) can be taught to allow the child to participate in age appropriate activities while still receiving instruction during school time on the standard math procedures necessary to complete such transactions.

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EDUCATION INTERVENTION: NEW APPROACHES

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The research generated by medical professionals has given us insight into the physical phenotype, molecular biology, cytogenetics, epidemiology, genetic counseling and inheritance of fragile X syndrome. Even with this information, the clinicians, teachers and related service personnel continue to seek effective treatments.

Fragile X females often present with learning disabilities, and traditional teaching techniques generated by the learning disability literature is usually effective. This paper will focus on some complex cognitive and educational needs of the male subjects. Most treatment methods were developed by clinicians in a search of a solution to educational problems. Little research, however, has been generated in the area of educational treatment. In order to plan successful teaching techniques, it is necessary to understand what is known of the cognitive function of fragile X males.

Table 1
Cognitive Features in Fragile X Males (K-ABC)
Kemper et al., 1988

N = 40
fra X = 20 (experimental)
children with developmental delays (DD) = 20 (control)

1. fra X IQ < than DD controls
2. fra X Achievement score > than DD control
3. fra X has more variation across subtests than DD controls
4. fra X Simultaneous (mean 71) IQ > Sequential (mean 62) IQ

The fragile X male usually has wide scatter among subtests on IQ evaluations. This wide scatter is part of the reason the males have been so difficult to serve in traditional special education settings, with traditional methods. Fragile X males commonly process information in a simultaneous fashion. This presents another challenge to educators who use traditional formats. For example, a simultaneous learner does not put individual parts together to make a whole, but rather requires the whole in order to understand its parts. Reading is most often taught in a sequential manner blending letters together to form words. The simultaneous processor will most likely fail to respond to that format and quickly lose interest. Instead, the simultaneous learner will see whole words or patterns inside words.

Fragile X males have demonstrated achievement ability that exceeds their cognitive ability. Kemper et al. (1988) noted that the fragile X males differed significantly from their developmentally delayed counterparts. There are surely a number of reasons for this, many of which have yet to be understood. In order to conceptualize this phenomenon, a cognitive phenotype has been created utilizing the findings from Kemper et al. (1988) and Dykens et. al. (1988).

Table 2
Strengths (+) and Weaknesses (-) in Cognitive Style of fragile X males

+ Long-Term Memory
+ Language Syntax
+ Achievement (Spelling & Reading)
+ Language Fluency
+ Simultaneous Processing
+ Matrix Analogy
- Sequential Processing
- Verbal Short-Term Memory
- Visual and Motor Skills
- Arithmetic
- Attention

On one side of the cognitive profile is a strength in long-term memory, but a weakness in short-term verbal memory. A number of researchers have examined the different memory functions related to long and short-term memory. The one that makes most sense in understanding the dichotomy in the fragile X cognitive profile is that short-term memory most often requires one to organize the information using a serial or sequential format. This would clearly present difficulty in the fragile X patient. The serial effect is not nearly as salient in the long-term memory. Long-term memory is a function of repeated exposure, thus capitalizing on the gestalt or simultaneous strengths noted in the literature.

Of particular importance is the organizational format necessary in the construction of memory. It is believed that if the information is organized into a coherent whole, each part is easier to retain (Hill, 1982). Such a framework is often referred to as a schema. Schemas can aid memory and also distort it. Many fragile X patients present information in a tangential manner, which often appears nonsensical and unrelated. An interesting dilemma is presented whenever standardized test results are analyzed. Due to standardized protocol, the examiner must follow carefully those responses deemed to be correct by the standardization sample. Many times the incorrect answer of the fragile X male seems far removed even from an educational guess. Those answers often reflect a distorted or disorganized schema that has been formulated in an individual who organizes globally and becomes easily distracted causing an interference with the initial exposure. Perhaps the speech patterns observed demonstrate what is most likely occurring in the process of organizing information into a distorted or disorganized schema (Scharfenaker et al., 1991).

Therapist:	Eli, tell me about your day.
Patient:	I like Hawaii, I like the hula.
Therapist:	Eli, how was your day? Tell me about it.
Patient:	It was okay; Luis, you're looking skinny.

In this case, it is not clear why Eli refers to his trip to Hawaii when asked to tell the therapist about his day. There may be no logical reason for it. Eli may have become so disorganized that his communication was truly unrelated in any way. On the other hand, there may have been a poster in the room that was tropical or the therapist could have reminded him of someone on his trip.

Another example of an association which may reflect a distorted schema often presents in the identification of faces and places in a subtest of the Kaufman ABC Battery. A picture of George Washington can often elicit a response such as money or dollar. The point being, even though the response appears inappropriate, it is the schema or organizational format that is creating the response.

Observation of responses on The Leiter International Scale of Intelligence indicated fragile X subjects more often succeed on those items that have meaningful context. For example, items related to matching on the basis of use, genus and analogous design rather than memory for series and gradation of shades of gray. Again, this may indicate a schema which is more global and simultaneous pulling on contextual organization rather than a sequential, serial or gradation form of organization.

An interesting question emerges. Does the gene dictate specificity in the formulation or distortion of the schema? Is there a mechanism that is consistent in the fragile X population? Is the organizational factor the same, but the experiences different? These questions should stimulate more research. Currently, data is being collected to ascertain responses from a variety of fragile X males. The subjects are shown drawings and asked to label them. It is hypothesized that those drawings will elicit similar schemas from a variety of subjects.

TREATMENT

Teaching to the strengths of this population is effective and successful. The additional confounding of performance due to anxiety and frustration from failure is as important as effective strategies. For example, to be expected to persevere through a sequential reading program that requires left to right sound blending can be devastating. Some of the time it can be accomplished, but with great frustration and negative feelings. It is much better to teach whole word, sight based reading programs. Often it isn't necessary to teach spelling, due to the advanced visual memory and ability to learn that kind of information incidentally.

Integrating a child's incidentally acquired knowledge into the curriculum should be the focus of education. Teaching numbers at a microwave touch plate, clock, keyboard, or license plate may be used in preference to traditional flash cards, work books, or number lines. The Logo Reading System (Braden, 1989) utilizes this incidentally acquired knowledge and encourages the learner to transform it into traditional formats (word cards, phrases, and writing sheets). This is just one example of an educational technique that utilizes simultaneous processing in the instructional process. The use of visual cuing with photographs, videotapes or Rebus pictures has helped in establishing pragmatic language. This method can be used to process the sequence of the day. The wholeness of the day's continuum serves to reassure the fragile X subject. The basic assumption behind visual pragmatism is that fragile X subjects need to organize into visual images. If the environment or academic information is vague, the subject will become disinterested and may become behaviorally disruptive. If visual images are provided, the success rate improves and the maladaptive behavior decreases.

It appears that the reason these techniques have proven successful is due to the inclusion of a component of meaning. This allows the fragile X student to formulate schemas which will provide them with success and reduce their anxiety. For example, providing verbal input or stories can give rise to success. If you want the child to color in a circle, the meaning is better illustrated if you ask the child to fill the pizza with lots of cheese. If you want a line drawn from point A to point B, tell the student to drive his car from home to school.

Curricular needs vary based on the functional level of the fragile X individual. Materials that utilize functional or real life materials provide interest and success. This allows the learner to organize incoming information into a familiar schema. Direct teaching often presents a confrontational relationship to the fragile X male. The use of a board game can often reduce the confrontational aspect of direct teaching and will more likely encourage spontaneous responses. Another technique that can promote spontaneous responses as well as encourage incidentally acquired knowledge is the use of age appropriate peers to provide models for responses. Teaching in a small group with non-affected peers allows the instruction to be cooperative (students asking the fragile X subject questions). This format reduces the anxiety that often precludes the fragile X student from attempting to organize and respond. The extent to which the anxiety factor interferes with responses has yet to be determined. Observational data implicates anxiety as a key factor to interference in successful educational experiences.

The educational arena presents a great challenge. Traditional methods are often ineffective and contribute to the deterioration of behavior and overall school performance. More research is needed to establish strategies of choice. Research to determine strategies, to validate a cognitive phenotype, to determine the extent to which anxiety interferes with learning and to establish the phenomenon of indirect and incidental learning ability are imperative in the search to define the fragile X learning style.

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SUGGESTED ACADEMIC MATERIALS FOR USE WITH FRAGILE X CHILDREN

1. Reading (sight/visually based)

   A. Logo Reading System by Marcia Braden, PhD, 219 E. Saint Vrain St., Colorado Springs, CO 80903
   The Logo Reading System utilizes well-known logos, traditional flash cards, sort cards with placements, phrase cards and matching/fine-motor work sheets.

   B. Edmark Corp., P.O. Box 3903, Bellevue, WA 98009-3903
   The Edmark Reading Program, level 1, is a beginning reading and language development program recommended for use with any individual who is considered a nonreader. The program contains 227 lessons presented in five formats: pre-reading, word recognition, direction book, picture/phrase matching and storybook lessons.

   C. SRA (Science Research Associates), Science Research Associates, Inc., 155 N. Wacker Dr., Chicago, IL 60606
   Corrective Reading Comprehension develops the reasoning processes (analogues, deductions, inductions, classification), vocabulary and writing skills students need. Reasoning is taught, not just practiced, in carefully written lessons that foster an

experience of success and self-worth.

D. Merrill Reading Series, Merrill Publishing Co., P.O. Box 508, Columbus, OH 43216-0508
The Merrill Linguistic Reading Program motivates students to become independent readers and encourages them to learn, to know, to think and to discover. The program offers readings in important areas such as science, health, history, mathematics and literature.

E. Appletree-Dormac, Inc., P.O. Box 270459, San Diego, CA 92128-0983
Appletree is an acronym for "A Patterned Program of Linguistic Expansion through Reinforced Experiences and Evaluations." It is a language system that provides sequential procedures for construction and development of the basic sentence structures that are the foundation of verbal language. The program has six workbooks, a teacher's manual and a pre-post test booklet.

F. Capture the Meaning, CC Publication, P.O. Box 23699, Tigard, OR 97223-0108
Here is an exciting new program that really teaches comprehension. Effective and easy to use, "Capture the Meaning: Strategies for Reading Comprehension" focuses on strategies for building comprehension. This 10-unit, 35 lesson program consists of teacher-guided instruction and practice, independent practice that includes individual and group activities, and tests -- all designed to reinforce and build reading comprehension skills.

G. Reading Attainment System, 1987, Education Design, Inc., 47 W. 13th Street, New York, NY 10011
This program is specially designed to supply practice for students who fail when basal tests reach the 3 - 5 grade reading level. Ease in reading comes only with practice at low reading levels. This system supplies that practice and produces fluency and confidence in students for whom ordinary methods of remediation have failed. Reading skills, vocabulary skills and thinking skills are offered in three different sets of multi-question exercises.

2. Spelling

A. I Can Print, Pro-Ed, 5341 Industrial Oaks Blvd., Austin, TX 78735
Designed to help students develop the necessary skills to form letters, begin writing sentences and build handwriting fluency, "I Can Print" can be used as a developmental program in kindergarten, first grade and special education. Also designed to be used as a remedial program for older children who have already learned to write but consistently have trouble with letter formation and spacing.

3. Mathematics

A. Logo Math I, by Marcia L. Braden, PhD and Ken Wood, PE, 219 E. St. Vrain

Street, Colorado Springs, CO 80903
The Logo Math Game utilizes well-known logos, board game, extra-large game pieces, dice, cards and spinner. Raised lines give sensory feedback as the piece is moved space to space to help teach 1:1 correspondence. The game can be used by three different level learners; matching, spinning and counting.

B. Good Apple Math Book, Grimm & Mitchell, 6 Apple, Box 299, Carthage, IL 62321
Math can be fun when students discover how to apply basic skills in practical ways. Activities are based on nature, mail order catalogs, popular foods, calendars, etc. Complete instructions for a math center with 28 idea cards and four game boards are included.

4. Social

A. I Can Behave, Pro-Ed, 5341 Industrial Oaks Blvd., Austin, TX 78735
"I Can Behave" revolves around an illustrated storybook comprising 10 stories and 125 full-page drawings. Each of the ten stories focuses on a specific classroom dilemma ("My Turn, Your Turn" - letting others talk; "Marvin and His Mouth" - using a quiet voice). Lessons including working independently, waiting for help, doing careful work, handling classroom frustrations and sitting still.

B. Social Skills for Daily Living, American Guidance Services Publisher's Building, P.O. Box 99, Circle Pines, MN 55014-1796
"Social Skills for Daily Living" presents a proven, effective method for enhancing the social skills of mildly learning-disabled, mildly emotionally disturbed, and mildly mentally retarded adolescents and young adults aged 12-21.

THE FRAGILE X EDUCATIONAL ASSISTANCE PROGRAM: AN OVERVIEW OF SELECTED PARTICIPANTS

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The Fragile X Educational Assistance Program was started in March of 1990 with funding provided by the North Carolina Council on Developmental Disabilities. The program is based at the Child Development Unit, Duke University Medical Center. The staff consists of a clinical child psychologist, a developmental pediatrician, and part-time consultants from surrounding communities who are knowledgeable regarding fragile X syndrome, including a speech and language pathologist, an occupational therapist, and an educational specialist. The purpose of this program is to "strengthen the educational services for children and adolescents with fragile X syndrome in North Carolina." Specific activities have included: 1) providing inservice training and workshops regarding fragile X syndrome for educators and other professionals working in the public school system; 2) participating in workshops for other health professionals throughout the state; 3) providing on-site consultation for specific students with fragile X syndrome regarding behavior management issues, educational programming, and IEP development; 4) assisting parents in securing an appropriate school placement and related services for their children, as well as developing an IEP or IFSP; 5) responding to requests for information and consultation via a toll-free telephone line; and 6) conducting psychoeducational, speech and language, and occupational therapy evaluations when this is useful in planning for a child.

Individuals have become involved in the program through referrals from parents, teachers, physicians and genetic counselors, as well as mailings and presentations to relevant professionals throughout North Carolina.

The following is a summary of our experience with 22 boys (ages 2 years, 8 months through 18 years, 6 months) in North Carolina. This may or may not be a representative sample of children with fragile X syndrome in North Carolina or any other state. However, we believe that it is useful to see how these boys are currently being served in the public school system.

Tables 1 and 2 summarize the available information regarding the most recent levels of cognitive and adaptive function.

Table 1
Most Recent Level of Cognitive Function

Borderline	1
Mild mental retardation	11
Moderate mental retardation	7
Not known	3

Table 2
Most Recent Level of Adaptive Function

Borderline	4
Mild mental retardation	4
Moderate mental retardation	6
Severe/profound retardation	2
Not known	6

Table 3 shows the diagnostic labels used by the school system to qualify the boys for services.

Table 3
Current Diagnostic Labels

Learning disabled	1
EMH	8
TMH	3
Other health impaired	5

Although the individual needs of the student are supposed to dictate placement, our experience has been that labels often drive placements. In other words, children with an EMH (Educably Mentally Handicapped) label generally attend EMH classes. However, boys with fragile X syndrome do not typically fit in these classes, nor do they do well.

Tables 4 and 5 show the type of placement and services being received by the 22 boys known to our program.

Table 4
Most Recent School Placements

Early intervention program (home-based services)
Center-based preschool program for exceptional children (2)
Developmental day care center
Montessori day care program
Readiness class
Multihandicapped class
Regular kindergarten class with support services (3)
Regular first grade class with one-to-one assistant
Learning disability, self-contained
Learning disability, resource class with some regular classes
EMH, self-contained

EMH, self-contained with one-to-one assistant
EMH/TMH self-contained (2)
EMH/Cross categorical, self-contained
TMH, self-contained
Homebound instruction

Table 5
Related Services

Speech and language therapy	21
Occupational therapy	15
Adaptive physical education	6
Physical therapy	2
Social skills training	2

Based on this information and our experience in North Carolina, the following points come to mind.

1. Parents, educators, therapists, and administrators need to be creative in considering school placement options for boys with fragile X. IQ level and the child's diagnostic label should be small parts of the equation and should not dictate placement. Rather, the individual needs of the child should drive the placement decision.

2. The speech and language needs of boys with fragile X syndrome are obvious, and services should begin as early as possible. For some boys, an augmentative communication system should be considered.

3. Even though occupational therapy with an emphasis on sensorimotor integration may not always be available, there are many benefits that can be derived from occupational therapy in general. Strengthening the child's fine motor skills and developing independence in daily living tasks are two important areas where an occupational therapist can be very helpful.

4. Most likely, the child's school placement will need to be reassessed each year. In making placement decisions, it is useful for parents to: a) speak with both the principal and the classroom teacher; b) visit any possible classrooms and schools being considered; c) attempt to measure the child's skills or progress in a meaningful way; and d) educate those teachers and therapists working with the child regarding fragile X syndrome. Some parents have prepared a notebook of selected articles regarding fragile X for their child's teachers and provided relevant videotapes as well.

5. Parents may also want to specify particular times to review their child's progress as part of the IEP (e.g., once per quarter, once per month, or even weekly if necessary).

6. Medication can be very helpful for boys with fragile X syndrome in terms of classroom function. Only 3 out of 18 boys in our group are not on some kind of medication.

7. Parents continue to be the most effective advocates for their children with fragile X syndrome. They are most likely to obtain needed services within the public school system, although support from knowledgeable professionals is often useful.

COMBINED OCCUPATIONAL AND SPEECH THERAPY PHILOSOPHY AND FUNDAMENTALS

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The Children's Hospital, Denver, Colorado

"Sensory integration is the nervous system's process of assimilating and organizing sensory information for functional use. An individual's ability to interact with the environment is influenced by how effectively and efficiently that person is able to process and use information from the tactile, vestibular, proprioceptive, auditory, olfactory, and gustatory systems." (Windeck & Laurel, 1989.)

Intersensory integration, or sensory integration, is integral to normal speech and language development. Sensory integration provides the crucial foundation for later, more complex learning and behavior. Sensory integrative theory and treatment are the result of the work of A. Jean Ayres.

The therapeutic approach consists of activities to facilitate the processing of information. The occupational therapist and speech therapist may choose to work together in a variety of ways. The models of intervention include consultative, observation, side by side, facilitative transdisciplinary approaches or individual therapy. The child often needs individual treatment sessions with an occupational therapist before he is ready for therapy in a small group with combined occupational and speech therapies. When the individual feels more organized and centered in his own body, he can be more receptive to the increasingly complex areas involving speech, social and academic skills.

A combined therapy approach in a small group is helpful. The stability, movement, rhythm, visual-spatial and tactile perception experienced in the body in the development of gross motor skill can be applied to the development of more rhythmic speech which is less echolalic and perseverative and more related to the child's actual experiences. The calming and centering provided by sensory integrative therapy can help the child focus on more cognitive, language related activities. Increased independence and self-control, more appropriate social skills in real life interactions, gradual acceptance of change and ability to sequence and make transitions are addressed at many levels, including gross motor, tactile, visual and olfactory.

Some important elements in combining therapies include:

- Knowing a child's functional levels
- Predictable sequence of a hierarchy of sensory integration
- Flexibility. Always respond to the child's every changing state.
- Alter physical environment as needed with music, equipment, room size, etc.
- Utilize well defined meeting space for children, with barrels, rug squares, bean bag chairs in a circle.

- Calming materials in center of circle available for children
- Use a "theme" for each session, one that is carried over across sessions as repetition is important
- Use no more than 4 activities in an hour.
- Start with a gross motor activity that incorporates deep pressure, weight bearing, touch.
- Move gradually to more fine motor activities.
- Breaks between activities need to be calming as well as alerting and preparing for the next activity.
- End session with quiet time to pull together activities, ex. singing about the hour's activities, preparing for the next transition.
- Sing directions, utilize rhythm in voice.
- Do not overload children with speech.
- Use simple language, at children's level.
- Model responses if children can't respond to questions.

One critical element is the "sensory environment." Therapists structure the treatment environment to help the child maintain appropriate levels of arousal and attention. This is something that should be done in the school and the home as well.

Some suggestions may include:

- Calm colors for background (light blues, lavender, pink)
- Natural and low lighting such as vita-lights (decrease use of fluorescent lights)
- Minimal auditory and visual distractions
- Use a study carrel instead of a desk to minimize visual distractions.
- Although many fragile X children will not tolerate earphones, they may be useful in decreasing auditory distraction.
- Well defined space boundaries through use of room dividers, tape on floor, carpet squares
- Carpeted barrel to crawl in; fabric tunnel
- Bean bag chairs

Have the following items available for the child to use if he/she needs it:

Vibrating pillow
Ace bandages for wrapping
Surgical brush for brushing
Weighted vests
Oral motor "chewy's" or toys
Ice chips to chew on

For additional information, please contact:
American Occupational Therapy Association
Sensory Integration Special Interest Section
P.O. Box 1725
Rockville, Maryland 20850-4375
(Phone: 301-948-9626)

Sensory Integration International
1402 Cravens Avenue
Torrance, California 90501
(Phone: 213-533-8338)

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Sarah Sharfenaker, MS, CCC-SLP
(Phone: 303-861-6800)

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ASSISTIVE TECHNOLOGY IN THE SERVICE OF COMMUNICATION

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Individually selected technology can assist many students with fragile X syndrome to communicate more capably. Augmentative and alternative communication (AAC) is a phrase referring to anything that helps or augments verbal communication. All of us use AAC whether we realize it or not – we gesture, use facial expressions and engage in other nonverbal behavior that adds to the communicative content of the verbal message. There are, however, more specific AAC techniques that are helpful to children with a variety of communication difficulties.

These difficulties fall into two categories. First, children or adults who have many thoughts and ideas to express may have verbal speech that is minimal or unintelligible or may have no verbal speech at all. There is a gap between their internal language level and their physical ability to express that language. Causes include hearing loss and neurological deficits affecting the oral musculature or verbal motor planning. These individuals require AAC in order to communicate at a level commensurate with their intellectual abilities.

A second group of individuals needs AAC techniques in order to better process and comprehend spoken language. These children and adults learn visual information much better than auditory information. They are helped when AAC techniques are used by their teachers, parents and friends, and by having those techniques available in their environments for their own use as well. Some of these individuals have verbal skills but have difficulty formulating and organizing expressive language. They may know the answers to questions but have trouble making an appropriate response. Word retrieval may be an issue. Dyspraxia, or problems with motor planning, may affect the individual to the extent that volitional responding is difficult. Visual cues, in the form of AAC techniques, provide cues to help the verbal language get started and organized. Children with fragile X syndrome often exhibit these language organization and word retrieval deficits.

TYPES OF AUGMENTATIVE AND ALTERNATIVE COMMUNICATION

Sign Language

One form of AAC is manual sign language. Signing can be very beneficial to children with fragile X syndrome, who often have delayed onset of speech. Some young children with fragile X syndrome are able to use signs to communicate basic needs and wants. Often, however, the poor fine motor and motor planning abilities of children with fragile X syndrome impair the ability to sign expressively. Sign language is more often helpful to these children as a cue for word retrieval and for organization of expressive language. Children with fragile X syndrome appear to learn the meaning of signs quite easily. Another helpful aspect of sign language is the use of sign pictures. Some children can interpret pictures of signs from a sign language book and use this printed cue to formulate a verbal response or as a stimulus item on a communication board.

Communication Boards

Communication boards constitute a second AAC technique. Boards may be either low technology or high technology. Low technology communication boards consist of pictures or symbols (such as the Mayer–Johnson Picture Symbols) arranged such that the child can point to one or more pictures to convey a message. High technology boards are "dedicated" computerized devices that add the component of voice to the communication board. Some devices, such as the WOLF by Adamlab, offer synthesized speech. Others, such as the IntroTalker by Prentke–Romich, have digitized (human) speech. Many types of voice output communication aids (VOCAs) are available in all price ranges. Individual assessments, considering a variety of factors, should determine which device is most appropriate for a specific individual.

VOCAs can be used with children with fragile X syndrome to help them organize a message visually before they respond verbally and to allow them to respond in situations that are extremely stressful. One child with fragile X used the WOLF to practice answering questions about a science unit on anatomy, a situation where he was very capable of mastering the content, but had difficulty demonstrating his knowledge verbally on command.

Facilitated Communication

Facilitated communication, which is currently receiving international attention, is the main focus of this report. It is an approach for identifying and facilitating use of language and cognitive skills for people who are nonverbal or who have limited verbal skills (Biklen, 1990). Its description is deceptively simple: A facilitator supports a person's hand, arm, or elbow to make it possible for that person to direct a finger to his chosen spot on a keyboard. The facilitator pulls the person's hand back immediately following his choice of a letter to prepare him to type the next letter. People who were previously considered to be severely intellectually impaired have been able to communicate by typing using this technique. Often, it is clear that they have taught themselves to read but need the technique of facilitated communication to express themselves. Calculator (1992) and McLean (1992) recognize that this technique is successful for a number of people; however, further research is necessary.

The following characteristics indicate likelihood of success with facilitated communication: nonverbal or nonfunctionally verbal (e.g., echoes what he hears rather than expressing himself), receptive language skills (e.g., "She understands an awful lot"), difficulty starting (follows some directions but not others that seem equally simple), difficulty stopping (perseveration), and difficulty with fine motor skills (e.g., "He uses some signs but doesn't seem able to move his fingers into position for many others").

Descriptions of Facilitated Communication with Children with Fragile X Syndrome

Facilitated communication has increased communication skills for all three of the nonverbal or minimally verbal children with fragile X syndrome to whom we have introduced it (in addition to more than 90 other children and adults with diagnoses of autism, cerebral palsy, mental retardation, or severe multiple disabilities). All three have intelligence test scores in the range of moderate to severe mental retardation, yet have demonstrated at least beginning literacy skills following a few weeks of practice with the technique of facilitated communication for both academic and social aspects of the children's school day.

Joey is 4 years old. His verbal skills consist of a few perseverative words and echolalia. With facilitated communication, Joey spontaneously types out single words to make choices and requests and responds to questions.

David, 11 years old and diagnosed as profoundly deaf, is nonverbal. He responds to communication through manual signs, but initiates only a very few signs, and these can be understood only by people who know him well and have learned to interpret his idiosyncratic attempts. Because David demonstrated no academic skills, he was placed in a class with six and seven year old deaf children rather than with deaf children his own age whose academic skills are closer to average. His teacher, trained in deaf education, attempted beginning academics with David for an entire year, with no evidence of success. Using facilitated communication, however, David demonstrated that he had been learning both reading and math skills. He has not initiated conversation by getting his word board or going to the computer, but when someone brings the word board to him or takes him to the computer, he frequently (although not always) responds to academic work, to (signed or written) questions about his preferences and to questions about the reason for appearing agitated or upset. David often types some meaningful responses, then a jumble of letters that cannot be interpreted. Writing out the meaningful words or letters followed by a blank space is often followed by his continuing the meaningful response. School personnel considered the program for deaf children inappropriate for David and recommended a program for severely retarded and multiply handicapped students. Based on his newly demonstrated abilities, however, it was agreed that a program for students with autism with a teacher with good signing skills who would receive consultation on working with children with deafness would better meet his needs. His teacher will also receive training in using facilitated communication.

Mike, 17 years old and diagnosed as having autism with intelligence test scores in the range of moderate mental retardation, often responded verbally to questions with one or two words. With prompts he might say a sentence, but he had not done this spontaneously. Using facilitated communication, he demonstrated some skills he has not performed verbally. Academic skills include naming coins (clearly though without perfect spelling, e.g., DM) and giving their value, doing addition facts, and selecting a word from a choice of three written words that either completes a sentence or answers a question. In responding to questions regarding preferences, Mike often types only the first letter of the word or only the consonants. This is not untypical of students who have never received formal reading instruction. A dilemma presented by Mike is that sometimes what he says and what he spells are not the same. For example, after consulting his picture schedule and telling his teacher (using facilitated communication to spell it) that he was going next to the cafeteria, she asked him what he would be doing there. He said, "Swim," but spelled LNCH. Typically, when this happens, what the person spells is what is intended while an unintended word or phrase seems to "pop out" verbally. However, most students who give verbal responses usually say what they intend to communicate. The dilemma is determining when the spoken word or phrase is intended and when an opportunity to communicate by spelling is necessary to determine the more accurate communication.

Equipment for Facilitated Communication

A laminated paper keyboard following either an alphabetical or typewriter keyboard pattern is a practically cost–free device for determining whether this technique has potential for a given individual. The disadvantage belongs not to the communicator, but to the communication receiver, who must remember the letters in order until the communication is complete.

An electric typewriter or a computer provides a visual and printed record. (Some people who use a computer for communicating require the screen to be covered. David, described above, could not communicate using the Apple computer in his classroom unless a cardboard covered the screen. Other communicators use the screen to identify mistakes in their spelling and correct them.) The disadvantage is lack of portability. It is certainly possible to use a combination of techniques. Thus, a typewriter or computer can be used for schoolwork and certain communications, while a paper keyboard can be carried everywhere.

Two portable electronic devices are currently available, and more are certain to be developed. The CresTalk™ is very portable and provides a readout of what is being typed, but there is no way to make an electronic print copy. The Canon Communicator is a portable device that prints a paper tape of what is being typed.

Many users of facilitated communication indicate preference for a specific device. Before investing in an expensive device, the user should have the opportunity to try a variety and determine a preference.

Responsibilities of the Facilitator

Certain behaviors by the facilitator (communication receiver) increase the likelihood that the communicator being facilitated will respond. Some guidelines follow:

1. Behave as if potential communicators have more cognitive ability than they have been given the chance to demonstrate previously. Let the person know this is the

expectation. Select reasonable, age and gender appropriate tasks that will be a challenge. Remember different individuals have different personalities, interests, and abilities. Discover these differences and respect them.

2. If disruptive behavior occurs (which happens infrequently with individuals with fragile X syndrome), tell the person you know he or she really wants to do the right thing. Suggest the person try to get control. If the person attempts to hurt you, say you know he or she doesn't want to hurt you and that you will protect yourself. Suggest the person try to relax and breathe slowly.

3. Keep yourself and your voice calm and relaxed. Individuals with fragile X syndrome (and those with autism) are likely to be highly anxious. Your calmness will help them remain in control.

4. Talk with an age-appropriate level of language. Don't use a "little kids" voice. Also, always include everyone present in any conversation. Never talk about one person to someone else when the person being discussed is present.

5. Find a chair and table that are the right height for facilitation. (Some people prefer to communicate with facilitation while standing up.) As the illustration shows, the individual's upper arm should drop straight from the shoulder. The lower arm should slant downward toward the keyboard. In "ready" position, the fingers are centered above the very bottom of the keyboard, so the communicator can see the entire keyboard and move forward and down to touch the desired key.

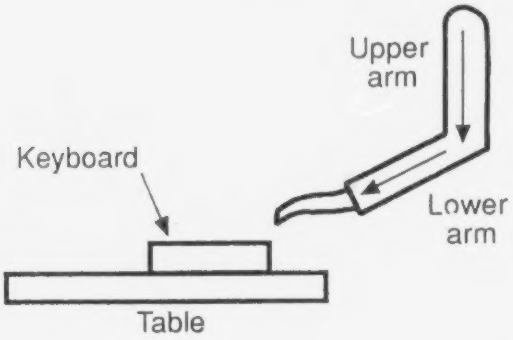

Figure 1: Proper seating for facilitated communication

A First Step Is Making Choices

A major reason infants develop communication is to make their preferences known. All people want to increase control over those aspects of the environment that affect them. Therefore, the opportunity to make choices provides a powerful motivation for an individual to respond to a new communication technique.

Following is a brief overview for providing choices in the areas of food and activities:

1. Choices should be given wherever possible (e.g., Do you want Cheerios or Rice Krispies? Do you want toast or a bagel? Do you want to go with Dad or stay home? Do you want to ride in the car or play in the park? Here are the kinds of juice we have. Which do you want? You can see the choices on this pop machine. Which do you want?). However, choices should not be given when the person does not truly have a choice (e.g., Don't ask, "Don't you want to go to Grandma's?" when the child must go with the family).

2. Give a list of two, three, or four things available to choose from. Don't give an open-ended choice. It may not be possible to fulfill it.

3. When the child is just starting to use facilitated communication, write the choices (or bring out items with names written on them), then guide the person's finger to each choice to focus vision as the word or phrase is spoken. This provides the spelling in case it is needed.

4. The facilitator and the communicator must both be positioned properly as described earlier.

Outcomes of Using Facilitated Communication

The technique itself has only recently been brought to national attention. Further, this attention has focused on its use with people with autism. Therefore, the use of facilitated communication with children with fragile X syndrome is too new and too limited to discuss outcomes at this time. The brief case studies described earlier suggest potential for augmenting communication skills and identifying academic skills for children with fragile X syndrome. The extent to which these skills can develop cannot be judged at this time.

As information and research on facilitated communication becomes available, children with fragile X syndrome with limited or no verbal skills should be considered as potential users of this technique for communication. Facilitated communication is a nonintrusive technique that treats the user with respect. In applying the principle of least harm, one notes that trying facilitated communication causes no harm. If it does not work, the person being facilitated has not been injured in any way. However, not trying it can do great harm if, in fact, it would have worked, because we are then depriving the individual of the opportunity to communicate and learn.

Using More Than One Communication Technique

Communication is crucial. The goal must be to determine the methods that best allow an individual to communicate. Seldom is there only one. Usually, there is a primary method, and additional techniques are used to augment that primary method. Sometimes, a particular method that has been used as the primary method does not provide the fullness of communication possible. When a new technique or method provides better communication, it should become the primary method, but that seldom means the original method should be discarded. It often continues to serve a purpose in some situations or with some people. For example, if a child's idiosyncratic signing has functioned to communicate certain important information to parents, this need not be lost when a VOCA proves to be a successful technique to extend communication. Sometimes techniques themselves must be adapted for a particular child. For example, some children are better able to communicate using a picture board when the technique of facilitated communication is used with pictures rather than with letters. Parents and speech/language pathologists need to work together to find the combination of communication techniques that allow the best expression for each child.

USING COMPUTERS FOR WRITTEN COMMUNICATION

Children with fragile X syndrome often exhibit great difficulty with handwriting because of the fine motor skill required. The handwriting itself takes such effort the child has no energy or concentration left to focus on the content of the lesson. When given access to a computer or electric typewriter for all written work, learning increases. (Many students function better at a keyboard using their own idiosyncratic approach rather than being forced into touch typing.) If learning to handwrite is considered important, instruction should be given separately from content instruction, so that all energy and concentration may rightfully center on the act of handwriting.

Related to this is the problem of copying from a chalkboard, even when a typewriter or computer is used. Many children with fragile X syndrome experience great difficulty in looking up at the chalkboard, then down at the keyboard repeatedly. A written copy next to the keyboard allows a child to focus on content rather than on the physical and visual requirements for copying. Here again, if teaching copying from a chalkboard is considered important, it should be done separately from content instruction.

CONCLUSION

Communication is a basic right for all people. With a willingness to try new techniques and use new technologies, the opportunity for communication can be made available to all children with fragile X syndrome. They have much to tell us.

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McLean, J. (1992) Facilitated communication: Some thoughts on Biklen's and Calculator's interaction. *American Journal of Speech–Language Pathology*, 1(2):25-27.

RESOURCES

CresTalk™: Crestwood Company, Dept. 9 2 S, 6625 N. Sidney Place, Milwaukee, WI 53209-3259. Telephone: 414 352-5678.

Canon Communicator: Canon, Inc., One Canon Plaza, Lake Success, New York, NY 11042. Telephone: 516 488-6700.

INTERVENTION TO ENHANCE ATTENTION SKILLS IN CHILDREN WITH ATTENTION DEFICIT HYPERACTIVITY DISORDER

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Institute for Basic Research in Developmental Disabilities, Staten Island, New York

Attention is a very complex process controlled by a large number of brain structures. Attention skills are either involuntary or voluntary. Involuntary attention (which is not a matter of conscious choice) is observed during the first few months of the infant's life. Involuntary attention occurs when the infant "is attracted by the most powerful or biologically significant stimuli" (Luria, 1973). The head turns in the direction of the stimulus as the eyes focus on the object. While the infant is behaviorally responding to the stimulus, physiological reaction is occurring as well. Orienting is considered an involuntary attentional process (Luria, 1973).

The development of higher or voluntary forms of attention occurs through the socialization process. The child's caretaker will name objects in the environment and point to them. The child's attention is thus directed through words and gestures. This is "a fundamentally important stage in development (which) later...gives rise to the type of organization of attention with the most complex structure, voluntary attention" (Luria, 1973). The process of voluntary attention takes years to develop. It begins in the early years at home and continues when the child receives socially organized attention training in school. The child does not immediately develop the ability to follow a simple verbal instruction to focus attention on a particular object. It does occur when the directive is simple and there are no other distracting objects in the visual field. "Give me the ball, please" can be followed by a two year old child so long as there are no other distracting objects around the ball. If an unfamiliar toy is next to the ball, the child will select the new toy instead of the one requested. "At this stage of development (roughly one year six months to two years four months) a spoken instruction cannot yet overcome factors of involuntary attention competing with it, and ... the direct field of vision " takes precedence (Luria, 1973).

The ability to follow verbal instruction becomes stronger at four or five years of age. Thus, the acquisition of language is directly related to strengthening this ability. The child learns to internalize the process of naming objects with the development of language. Speech enables the child to make more selective responses. Luria (1973) indicates that the development of voluntary attention occurs at age twelve to fifteen.

The components involved in the process of attention are biological, social and individual. Each individual matures at his/her own rate which interacts with his/her unique genetic make-up. In addition, the process of attention relies heavily on social and interactional development and exposure to the environment. Attention deficit is believed to be one of the cardinal manifestations of Attention Deficit Hyperactivity Disorder (ADHD). Interestingly, it is not understood completely how the process of attention works and what specifically is the nature of the deficit in those diagnosed with the disorder. There are different functions of attention. A person could experience difficulty with any one function, a combination, or the entire process. In addition, attention difficulties can be a symptom of any number of disorders. Consequently, researchers have not been able to demonstrate distinctions between ADHD attention deficits and those of other etiologies.

The primary or essential features of ADHD are attention deficit, impulsiveness and hyperactivity as stated in the DSM-III-R (APA, 1987). Low self esteem, low frustration tolerance, inability to delay gratification, aggression or other emotional disturbance and poor social development are considered to be secondary symptoms of ADHD (Conners & Wells, 1986; and Loney, 1980).

Hyperactive and impulsive behaviors develop into behavior problems. The children exhibiting them are readily identified by teachers and parents as these behaviors are often very disruptive. The term hyperactivity refers to an excessive, high level of activity that persists over time. It is excessive movement that goes beyond an acceptable limit. This excessive movement appears to be non-goal directed. The child is constantly moving, unable to be still or stay seated or stay in one place, fidgets, talks incessantly and appears to have inexhaustible energy. The child is restless and often appears agitated or anxious. Hyperactivity is not considered to be a movement disorder as such because the movements, although excessively performed are normal (Lohr & Wisniewski, 1987). In other words, there is not the gross or fine motor dysfunction that exists in cerebral palsy or Parkinson's disease. Impulsive behavior is exhibited when one acts before considering the consequences of the action. Additionally, one shifts from one activity to another excessively, has difficulty organizing work tasks, requires more supervision than peers, inappropriately calls out in class, and/or has difficulty playing fairly (i.e., waiting for a turn). Generally, a low level of frustration tolerance accompanies these symptoms. Since frustration is associated with dissatisfaction from failure of goal attainment, low frustration levels would make goal attainment virtually an impossibility. Intimately connected with low frustration tolerance is the inability to delay gratification.

Hyperactive children have problems with peer relationships and social development. Hyperactivity is usually complicated by attention deficit which may result in an insensitivity to social feedback cues. The impulsive nature will lead them to say things at the wrong times. It appears "they exhibit an inordinate need to inspect, manipulate or provoke their peers" (Levine, 1987). The result is often peer rejection. These children usually have no idea why others reject them. Consequently "hyperkinetic [hyperactive] children develop a coping style which is often destructive to interpersonal relationships" (Lubar, 1985). Aggression is one strategy the hyperactive child can develop in order to express the frustration from lack of goal attainment and peer rejection he/she experiences. The majority of hyperactive children, in most clinical settings, are also aggressive (Loney, 1987). This being the case, it is a confounding problem for people who have to deal with these hyperactive-aggressive children.

Some researchers have stated that aggression is a logical consequence of the low frustration tolerance and impulsivity seen in hyperactive children (Laufer and Denhoff, 1957). Typically, the overly active child becomes hyperactive, then oppositional. One could speculate further that the oppositional child can become aggressive. The overly active child moves from hyperactive to oppositional to aggressive as time passes and emotional difficulties are not resolved. Thus, we may be seeing an intensity of unresolved emotional disturbance, manifested in behavior, that progresses through continued maladaptive strategies, to an aggressive behavior disorder.

There have been many reports demonstrating that males with fragile X syndrome (fra X) are subject to hyperactivity and impulsive behavior. Sometimes the hyperactivity and impulsive behaviors have been associated with poor perception and integration of information (Hagerman et al., 1985). Fryns et al. (1984) have suggested that hyperactivity is very characteristic of males with fra X, who often have concentration difficulties. Other authors have also reported short attention span and increased distractibility in males with fra X (Largo & Schnizel, 1985; Hagerman et al., 1985; Levitas et al., 1983). Bregman et al. (1988) studied 14 clients (3-27 years of age) who ranged in intelligence from nonretarded to moderate retardation. Information was gathered from parental interviews, medical and psychoeducational reports, the Conners Parent and Teacher Questionnaire and the Child Behavior Checklist. All 14 males with fra X presented with inattention and impulsivity and 13 of the 14 clients met the DSM-III criteria for ADHD.

A number of intervention strategies (e.g., behavioral modification, cognitive skills training, medication therapy) have been used with children who have ADHD. These methods have all had short term positive effects. The problem traditionally has been achieving long term positive effects on enhancing attention and concentration skills and the reducing of hyperactive and impulsive behaviors.

The efficacy of neuropsychological training in enhancing attention and concentration skills with males with fra X is currently being investigated. Neuropsychological training was originally developed to facilitate the rehabilitation of brain function lost through injury. Following trauma, the natural ability of the brain to compensate and recover lost function begins. There can be considerable recovery of function. The apparent plasticity of the central nervous system provided the basis for the development of training techniques to assist and enhance the brain's natural recovery process. Craine and Gudeman (1981) reported that the primary objective of neuropsychological training is to assist and enhance the developmental processes underlying learning. Theoretically this training will benefit anyone. It is mentally exercising the brain. Just as our bodies benefit from regular physical exercise, our brains benefit from mental exercises. Generally our mental stimulation is accomplished through education, learning a skill or hobby, or pursuing some endeavor which challenges us to arrive at solutions. However, some may need an extra push in the right direction. It is believed that children with attention deficits, with or without hyperactivity, will benefit from neuropsychological training, as it may be the extra push they need to develop the skills they lack.

What is presented below is a neuropsychological training program that is currently being used with a male with fragile X syndrome. Details of the training and the objective of each of task being used with the child are provided.

NEUROPSYCHOLOGICAL TRAINING PROGRAM

Training sessions are conducted twice a week for forty minutes each session. During a forty minute session the child works on three of the five exercises (described below). The selection of exercises and the pace is determined by the trainer. Training regimens are geared for successful completion. The trainer records session dates and time lengths and the training progress for each training session. The five exercises selected are: Ace-to-King, Attributes, Master Mind, Simon and What's in the Square.

Ace-To-King: A standard deck of cards is used. The trainer sorts the deck and works with the one suit the child chooses. Each picture card is given a numerical value (ace = 1, jack = 11, queen = 12, king = 13).The initial trial begins with three cards. The trainer demonstrates the activity for the child. Following the demonstration, the cards are shuffled and placed face down, in a row in front of the child, before giving him the instructions which are as follows:

> In front of you are (the number) cards, face down so you can not see the order. The cards are not in order. Turn the cards over one at a time, say what the card is out loud. Remember where each card is. When you reach the end of the row, turn the cards back down. When I call for the number, turn the right card over in order (e.g., 1,2,3...). Now, before you start, tell me what you are going to do. The child then repeats instructions in his own words, and trainer repeats if necessary. The criterion is perfect recall for each trial, two consecutive trials, before adding the next card.

The objective is to increase attention, concentration skills, memory and ability to organize.

Attributes: (Creative Publications, Inc. 1976). Activity consists of six colored circular strings, 32 blocks (four shapes, circle, square, diamond and triangle; four colors, blue green red and yellow; and two sizes, large and small) and stimulus cards.

All of the blocks are placed in front of the child. Initially one ring is placed on a table in front of the child. A single stimulus card is placed inside the ring. For instance the stimulus card could contain the color red. The child must then place all the blocks that are red inside the circular string. This task has several levels of difficulty and culminates with the child working with three rings and three different stimulus cards having to sort the blocks paying attention to seven different combinations according to the appropriate attributes. The criterion for advancing to the next level of difficulty is successful completion (no mistakes) of all tasks in a set of cards for two consecutive training sessions. The instructions are as follows:

> I am going to put a card in the circle (or, each circle). I want you to look at it (them) and tell me what you see. (Child correctly responds.) Very good. Now put everything in the circle(s) that the card(s) tells (tell) you. Check your work when you finish and let me know when you are done.
>
> After completion:
>
> Now tell me how you decided to put these blocks in the ring(s).

Development of logical thinking, problem solving and planning abilities, attention and concentration are the objectives of Attributes.

Master Mind: (Pressman Toy Corporation, 1981). This activity consists of the Pressman game board, six different colored code pegs and two different colored key pegs (white key peg indicates that the color of the selected peg is correct; black key peg suggests color and position of the selected peg is correct). The trainer selects four colored pegs and places them in the code box out of view of the child. The child then must try to break the code and discover the choice of color and/or position of the four colored pegs. There are three ways to play the game, of varying degrees of difficulty. First the child is given the four color pegs and he has to place them in the correct position (position only). Second the child has to solve the problem by selecting the correct colors of the pegs (color only). Third the child has to solve the problem by using color and position (color and position). He is taught to utilize nonverbal feedback to problem solve the code. Criterion is met when the child demonstrates he is using feedback information and breaks the code in a minimum of four trials.

The objective is to develop a problem solving approach through hypothesis formation and immediate feedback.

Simon: (Milton Bradley, 1984). The Simon game is battery operated. It has a round black base with three selection switches and three command buttons. Surrounding the selection switches and command buttons are four large colored lenses (red, blue, green and yellow). Instruction are as follows:

> This is a game called Simon. It is like the game 'Simon Says.' Do you know how to play 'Simon Says?' (If the response is yes, continue with instructions; if no explain the basis of the game.) I'm going to press the red button and Simon will light up one of the colors. Then you will have to do what Simon says and press the same color. If you pressed the right color, Simon will repeat that color and add on more. You'll have to press the colors in the same order as Simon has given them to you. Continue to repeat Simon's signals as many times as you can. Simon will let you know when you make a mistake.

Thus as is readily seen, visual and auditory stimuli are produced for the player to follow sequence. Criterion is to pass each level (which is built into the game).

The objective of Simon is to develop ability to remember a spatial/sequencing pattern when using auditory and visual feedback.

What's In The Square: (Stachel, 1973) This activity consists of the game board, which is divided into 16 squares and 10 sets of cards. Each set of cards indicates a level and within each level there are many stages to be accomplished. Additionally each set of cards has its own border cards. The board is placed in front of the child. The border cards are placed on the board, each heading a row on the matrix. The trainer identifies what the rows are (e.g., this is the row for all the things that are blue, or this is the blue row). The deck of cards is placed upside down in front of the child. Working from the top of the deck, the child selects a card, tells the trainer what it is and places the card in the correct box on the game board. The instructions are as follows:

> This is What's in the Square. Here are the border cards. They tell you what belongs in each row. Can you tell me what belongs in this row and this row...Very Good! (If the child does not know what belongs in what row, then the trainer tells the child.) Now here is the deck of cards. Turn them over one at a time, look at the card, tell me what it is and then place the card in the right box. Continue to do the same with all the cards until all the boxes are filled. Criterion for advancing to the next level is completion of tasks without mistakes.

The objectives of this activity include: develop the ability to distinguish between objects according to characteristics such as color, size, shape, etc.; identify the common property to a set of objects when sorting; visualize an object belonging to two groups simultaneously; combine and separate objects into subgroups; find various ways to solve a single problem; and verbalize and understand concepts and characteristics.

NEUROPSYCHOLOGICAL TRAINING WITH A YOUNG MALE WITH FRAGILE X SYNDROME

The fra X male is six years old. He has a full scale IQ of 87, a verbal IQ of 97 and a performance IQ of 69 as measured by the Wechsler Pre-School and Primary Scale of Intelligence (WPPSI). Academic skills are at the pre-first grade level as indicated by the Wide Range Achievement Test Level I (WRAT-R). He has articulation difficulties. The goal of the training program is to enhance his attention and concentration skills thus allowing him to learn better in school.

He has been receiving neuropsychological training twice a week for forty minutes each session since the end of March 1992. This young male has been exposed to all the training exercises. What follows is a summary of his progress to date. Please be reminded that the child only receives 3 activities per training session. Thus the activities have not been presented an equal number of times. He has worked with Ace-to-King 22 times, What's in the Square 21 times, Simon 19 times, Attributes 18 times and Master Mind 3 times.

Ace-to-King. He liked this activity the best (as would be expected since males with fra X are known for their good memory). He started with three cards and quickly worked up to five cards by the second session and three sessions later he worked on six cards. However, in order to meet criterion for seven cards it took this young man two months, indicating seven cards was a large load for his memory. He is currently working to meet criterion with eight cards.

Attributes. By the seventh session, he was working on two rings overlapping with a single stimulus card in each ring. This activity is taking this young man relatively longer than Ace-to-King for he is being asked to slow down his movements so as to curtail and control his impulsive responses. He has not progressed beyond this level within the four months of training.

Simon. Simon was the third activity presented to this young man. The concept of Simon was very difficult for him to master. He has worked slowly with this activity but continues to progress. This is to be expected as this activity relies heavily on being able to pay attention to the visual stimuli. He started with a total score of two correct responses in a row and is currently able to attain a score of 4 correct responses in a row.

What's in the Square. This activity was introduced in the second session. He has accomplished the introduction level of all ten decks of cards. This means that he has mastered the basic principle and can place the correct cards in the correct rows. It is noteworthy that he had difficulty counting prior to training. However, Ace-to-King and Deck 10 of What's in the Square require counting which he is able to do now. By the seventeenth session, he started the advanced level of deck 1 and by the twenty-first session he was working in the advanced level of deck 2.

Master Mind. He has only received Master Mind three times. He was introduced to Master Mind twenty sessions into the neuropsychological training. He is currently just learning how to play the game.

We are still early in the neuropsychological training. However as indicated above, this young man has demonstrated progress through the different levels of difficulty with a number of the activities. Additionally, he has begun to count. Neuropsychological training may very well provide an intervention that could possibly improve attentional deficits in children with fragile X syndrome.

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PRACTICAL CONSIDERATIONS IN PROVIDING PSYCHOTHERAPY FOR FRAGILE X INDIVIDUALS

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Given the myriad and complex symptom picture that the fragile X individual may present, one of the critical and often difficult questions revolves around when to refer for psychotherapy. There are many ways of approaching this issue, just as there are many approaches to psychotherapy (Brown et al., 1991). One general approach that has been found helpful in resolving this dilemma is the developmental approach. The developmental approach recognizes the psychosocial context in which the person develops and also emphasizes that there are periods, i.e., stages, during which the tasks or demands facing the individual changes. The point at which demands and expectations change is recognized as potentially being more stressful and, therefore, having greater impact on the individual's adjustment. Therefore, one would expect that the fragile X individual might experience significant adjustment difficulties during periods of transition and should note the extent to which their adjustment deteriorates and seek appropriate help. Additionally, major disruptions may be observed in the individual's level of adjustment if significant difficulty is encountered in achieving mastery in areas that contribute to their sense of independence, competence and self-esteem at any stage.

The information provided at this conference has ranged from highly specific and technical cytogenetic studies to less specific research involving adjustment and psychosocial functioning. This presentation focuses on attempts to enhance the psychosocial functioning of the fragile X person through the use of psychotherapy. Identifying the characteristics/symptoms associated with this syndrome, their significance and origin has been a significant part of research efforts. It is equally important that psychotherapists recognize and understand the significance of characteristics associated with the fragile X syndrome as they affect the individuals' function and as they may influence the therapeutic process. In order to facilitate this understanding, a brief overview of some characteristics associated with the fragile X syndrome and consultations that may be helpful to psychotherapists will be presented.

It is important to remember that a particular person may not display all of the characteristics that will be discussed and that one should guard against what Reiss et al. (1982) called "diagnostic overshadowing," i.e., the tendency to attribute all of the person's symptoms to the fragile X syndrome once this diagnosis has been made. Ultimately, each person must be dealt with on an individual basis and their particular strengths and weaknesses determined during the process of trying to enhance their capacity to function effectively.

CHARACTERISTICS

Hypersensitivity to Stimulation - Visual, Auditory, Olfactory and Tactile:

It is important to understand to what stimuli is the person reactive as well as what stimuli may have a calming effect on the person. Occupational and speech and language therapists may provide helpful feedback regarding the significance of the person's reaction to certain stimuli as well as information about calming and relaxing techniques that may be helpful to the therapist.

Difficulty with Motor Planning and Sequencing:

This area is particularly important as it affects the person's capacity to follow instructions and implement suggestions and recommendations. Consultations with occupational and speech and language therapists may prove helpful in understanding the nature and extent of the difficulty the person may be experiencing, as well as information about how the therapist may be able to circumvent or lessen the difficulties associated with this.

Attentional Difficulties, Hyperactivity and Impulsivity:

The prevalence and variability of these characteristics necessitate consultations with educational, vocational, and residential personnel and parents to determine the nature and range of situations in which these situations exist as well as provide feedback to them regarding ways the environment may be structured to reduce the impact of these problems. It may also be necessary to seek medical/psychopharmacological consultation to assist in the management of these problems if other efforts are not successful.

Difficulty with Transitions:

These difficulties may involve changes in routines, shifts from one activity to another or even changes involving conversational topics. Speech and language and occupational therapists may provide useful information regarding ways to structure and facilitate changes and it may be important for the therapist to consult with others about ways to enhance smoother transitions through the combination of anticipatory planning and the use of relaxation techniques. It also may be useful to consider a psycho-pharmacological consult if significant agitation and out of control behaviors are associated with these situations.

Difficulty Negotiating Social Interaction:

This may involve social avoidance or a variety of other problems that result from difficulty understanding and/or using social cues and lead to fragile X individuals experiencing problems in social situations. This may require the therapist to consult with a variety of personnel involved with the individual in order to fully appreciate the problem. It will also be necessary to integrate this information and provide feedback to others about ways to reduce the problems in this area. Consultations from occupational therapists regarding the use of self-calming and relaxation techniques can be useful.

Communication Difficulties:

Receptive and Expressive Language, Perseveration, and Topic Maintenance: Psychotherapy, in large measure, relies on the verbal medium as a means of communication and transacting the therapeutic process. This is, however, one of the areas in which fragile X individuals may have considerable difficulty involving distortions which affect what they take in verbally or be able to express. Consultations with speech and language therapists may be especially helpful in contributing to knowledge regarding the level of comprehension the person has as well as in providing suggestions about the pattern of speech that are likely to facilitate communication.

Developmental Level and Psychologic Differentiation:

This involves the person's level of cognitive development and psychosocial stage of development, as indicated by the nature of their emotional and social concerns, as well as the behavioral, adaptive and social skills manifested by the person. Consultations from other allied medical professionals and psychological assessment are important in helping to determine these and in establishing the direction and focus of treatment. These may also affect the extent to which structure may be useful in therapy as well as the type of therapy that might be effective.

The characteristics and associated consultations noted above are examples of critical factors to be considered when providing psychotherapy to fragile X individuals regardless of the therapeutic approach used. While these were described to assist psychotherapists in treating the fragile X client, it is recognized that it is easy to become overwhelmed and/or preoccupied with the symptom behavior manifested by fragile X individuals and, in the process, overlook the psychosocial aspects of their function. To be sure, behavior must be assessed to determine the extent to which it interferes with adaptive function. However, the goal of psychotherapy, regardless of the approach, is to facilitate the adjustment of the individual in such a way that it enhances the capacity of the individual to cope with new situations as they are encountered. This may involve engaging the child and/or adult in individual and/or group psychotherapy. It may also involve engaging the family of the fragile X individual in treatment in order to facilitate their adjustment as a family. Regardless of the approach to psychotherapy and the level of intervention, the greater the degree of familiarity and comfort the therapist has with developmental disabilities the greater the likelihood of effective treatment.

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IN-DEPTH PSYCHOTHERAPY WITH PEOPLE WHO HAVE FRAGILE X

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INTRODUCTION

The screaming continued for more than twenty minutes. The classroom looked as if a tornado had just passed. Corey[1], a 6 year old boy, was sitting in the corner. His beautiful blue-green eyes were filled with tears. He looked around with astonishment and disbelief. It was hard to comprehend all of this was as a result of his tremendous outburst.

Caregivers who work with children and adults with fragile X know how stormy such outbursts can be. What can one do in such a situation? How should a caregiver deal with a person who has just gone through such an experience? What is the "right" treatment approach?

Most often, such questions are answered in behavioral terms with concrete solutions to such situations. Usually when the origin of a condition is in the physical realm, research and treatment tend to emphasize its biologic aspects. Thus, focus is put on a search for answers and solutions in the physiological, biochemical, cellular, molecular, etc., fields. Similarly, developmental, educational, psychological and behavioral manifestations are also explored in this fashion and treatment is designed in concrete terms of remediation and behavioral management.

In the past, in-depth psychotherapy, especially with people who have developmental handicaps and/or severe behavioral difficulties, was not considered an acceptable treatment approach. However, lately, some openness has emerged among professionals with respect to the potential of psychotherapeutic approaches in the treatment of such populations. In this paper the author will demonstrate the importance and success of the use of in-depth psychotherapy with people who have fragile X. It will be shown how, through the use of a psychotherapeutic rather than managerial approach, the emotional stress experienced by individuals with fragile X can be relieved and growth and change can occur. The utilization of a variety of psychotherapeutic modalities and their impact on the clients will be demonstrated through case illustrations.

Understandably, identifying the cytogenetic and physical characteristics and symptoms associated with fragile X is an important part of the research in this field. It is also important to recognize and comprehend the emotional and psychological aspects of the condition because they too affect the individual's function. This paper will not elaborate on the first aspect but will rather focus on the latter and identify those manifestations which are relevant to the emotional-psychological characteristics of people with fragile X and are, therefore, influential in the psychotherapeutic process. Through the use of case illustrations, emphasis will be put on the emotional and psychological characteristics typical of people with fragile X. These illustrations will also elaborate on the change and growth that occurred as a result of the psychotherapeutic process.

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[1] The names and some identifying details were changed to protect the identity of the people.

FIRST ILLUSTRATION

Lisa, Mary and Kay are three sisters who, only after many years of psychotherapeutic treatment, were diagnosed as fragile X. The oldest, Lisa, was in treatment with a male psychiatrist since 9 years of age. She was transferred to the author's care at the age of 18 because it was felt that she would benefit from a woman psychotherapist. For 3 years Lisa found it extremely hard to accept this change. During this time she exhibited anger, impulsivity and mistrust. Lisa accused her parents and the therapist for the separation from "her" psychiatrist and she was extremely resistant to becoming involved in a new therapeutic relationship.

This difficulty in the formation and establishment of relationships was clearly evident also in Lisa's interpersonal interactions outside of the therapeutic realm. Lisa did not know how to appropriately relate to people. She was very scared to form any relationships both because of her lack of social knowledge and her fear of people which she had projected onto every person she met. Until she was 27, Lisa was able to befriend only one girlfriend and had never experienced a friendship with a man. Similar to many people with fragile X, she experienced great difficulties in the perception and comprehension of social cues. As a result, she was unable to acquire the social knowledge needed to negotiate interpersonal interactions.

In her psychotherapy, even when she allowed herself to communicate verbally rather than to sit silently, Lisa was unable to engage in in-depth processes. She did not remember dreams and was unwilling to allow herself to experience any therapeutic modality (imaginary, art, movement, etc.) other than verbal therapy.

It took a long time to gain Lisa's trust. It took even longer before she allowed herself to recognize her potential even though she has difficulties. This emotional change became apparent in her behavior. Lisa learned to talk in a more pleasant voice rather than in the previous whining tone she often used. She was more willing to listen and to take the risk of hard work that leads to change. She acquired enough self confidence to go back to school and not only upgrade the credits for her high school diploma, but also graduate with a college degree in Early Childhood Education. To practice, she worked as a volunteer in a day care and started to think of finding a position in the competitive market. However, in spite of these achievements, her difficulty in negotiating social situations continued to affect her and she was unable to hold a paying job for more than a few months at a time. Although very upset each time she was let go, she did not fall apart and after a while would continue to search for another job.

In the case of people with fragile X, progress and change take a long time. Seven years into her therapy, Lisa started to contemplate the notion of meeting a man and forming a relationship. At that time she was able to take risks and "practice" relating to people, especially to men. A few years later, through an arranged introduction she met the man who would later become her husband. She is now married and is a mother to two children.

Lisa's sister, Mary, started her therapy at the age of 24. She suffered from difficulties similar to those experienced by Lisa. Mary, however, was a lot more aggressive in the way she related to her human environment. Lisa learned to cope with life by covering up her difficulties through avoidance and anger; Mary learned to relate to her environment by way of verbal abuse. She behaved in this aggressive manner each time she tried to cope with the complexity of social situations and became very compulsive when trying to participate in interpersonal relationships.

In addition to the above, Mary also exhibited attention deficits, hyperactivity and impulsivity. As a result, while a student in high school, she was assessed by the school system and diagnosed as having a non-specific learning disability. When she started treatment, she had already experienced failure in finding and holding a job, which effected her attempts to look for and find work.

In her therapy, Mary allowed herself to work on a deeper emotional level. She worked through her anger and utilized dream work and body work. She learned to accept her physical appearance and understood that it was possible for her to take better care of herself. Her greatest achievement manifested itself in a better acceptance of herself. She also started to forgive herself for her mistakes. This resulted in a decrease in her abusive outbursts and increased her tolerance of changing or ambiguous situations. Eventually, Mary took the risk of going back to school. She took a secretarial course in a business school and was able to find a job which she still holds five years later. Due to her difficulties in understanding and appropriately behaving in social situations, she needed to learn and practice how to behave in a work place and how to relate to her co-workers. It took about a year for Mary to learn the social skills she needed so as to avoid losing her job. A year after establishing herself in her work place, Mary stopped her therapy.

Kay, the third sister, began therapy as a result of an emotional trauma. Although she is more quiet than her two sisters, Kay, too, has experienced similar emotional and psychosocial difficulties to those described. In addition, Kay also suffered from a variety of phobias which, more than in her sisters' lives, caused her tremendous functional hardships.

As in the case of her two sisters, Kay has also developed her own ways of coping with challenging situations. Kay, who felt very intimidated by parental figures, would either cry to make people take care of her, or lie to avoid what she experienced as her failure to answer expectations. In addition to her learning disability, Kay, more than her sisters, also suffered from great confusion. She experienced difficulties in comprehension of sequences, which affected her ability to plan even simple tasks. Simultaneously, she resisted following instructions and implementing suggestions because she perceived it as a confirmation of her impairment.

The psychotherapy with the three sisters and the occasional consultation with the parents made me realize that all of them (mother and her three daughters) suffer from very similar difficulties. The nature of the deficiencies in the emotional, psychological, social and cognitive areas put it beyond "nurture" (i.e., environmental effects). A thorough cytogenetic assessment revealed that all of them had fragile X syndrome, although the degree to which they were affected varied.

This discovery gave the family some answers which helped to make sense of what, until then, had no logical explanation. In spite of the devastating diagnosis, this new knowledge brought with it some relief and enabled the parents and their daughters to work through their anger and feelings of failure which all members of the family shared.

A powerful emotional denominator, common to the mother and her three daughters was the very high level of anxiety experienced by them each time a somewhat unusual situation presented itself. Although the level of their cognitive function differed (the mother was the most adjusted one), the effect of this emotional characteristic created, time and again, a crisis experience which led to feelings of helplessness and disintegration.

As a result, symbiotic interpersonal relationships evolved in the family. The parents had great difficulty in letting go of their daughters and in enabling them to become more independent. They feared for them and tried to protect their daughters from the hardship they had experienced in their lives. Lisa had grown up to become an extremely secretive and suspicious person who avoided sharing any information with others. Mary had grown up learning to react in an aggressive way through which she pushed away any "intrusion" and Kay had become even more dependent and incapable of making any decisions without involving her mother.

In her psychotherapy, Kay allowed herself to utilize psychotherapeutic modalities other than verbal. She was able to remember dreams and work through some of their symbolic meanings. She joyfully participated in psychodramatic play. She painted and wrote poetry. In Sandplay Therapy, which is an in-depth Jungian orientated modality, created by Dora Kalff (1980), Kay worked, among other things, on her very deep need to be dependent on her parents as well as her perception of herself as a little child within the family.

Sandplay Therapy is conducted in a room in which there are two sand trays (one with wet sand and one with dry sand), and hundreds and hundreds of various figurines depicting the world. The person chooses figures from the presented figurines and creates a picture in the tray of their choice. The pictures are expressive of a deep, unconscious and archetypical level and symbolically bring to the surface the innermost psychological constructs and emotional structures. Through the therapeutic process these emotional issues are explored and a re-constellation of the Self occurs (Weinrib, 1983).

Kay was able to use Sandplay Therapy well. Kay's therapy focused on clarifying her reaction patterns to confusing and complex situations; on developing more efficient coping mechanisms; on learning to tune in to her emotions and, thus, on obtaining a better understanding of who she is; and finally, on exploring ways to become more autonomous.

SECOND ILLUSTRATION

There are other emotional manifestations of the various cognitive, perceptual and intellectual difficulties that people with fragile X experience. One of the most difficult, for both the person with fragile X and the caregiver, is the explosiveness which might occur without any "apparent" reason.

Eliot was a big and heavy 15 year old boy who constantly expressed his anger and frustration in extremely aggressive behavior. In his violent outbursts he did not differentiate between objects in his physical environment and people in his human environment. When such a violent explosion occurred, Eliot needed to be contained so that he would not hurt himself or others. This issue of containment is a central issue for many people with fragile X. Eliot had great difficulty in creating boundaries. He dumped hundreds of figurines into the tray without discrimination. The flooding effect which he created is an accurate and concrete manifestation of the rush of emotions within him which he could not contain and which pushed him to explode. One might relate to such explosions solely on the behavioral level and create management programs in order to eliminate the behavior.

Psychotherapeutically, however, if we understand the meaning of this behavioral phenomenon, and if we comprehend the tremendous stress and inability to cope that is communicated through it, we might be able to provide the person with a humanistic container within which new emotional structures will be formed and new modes of communication will evolve (Baum, 1992).

THIRD ILLUSTRATION

Danny is an eleven year old boy who started to attend the Muki Baum Treatment Centre six years ago. He came to the Centre with a confirmed diagnosis of fragile X and therefore could be attended to in a more direct and appropriate manner initially.

Danny presented an extremely explosive pattern of behavior. He would become both verbally abusive and physically violent toward anyone in sight. He very clearly demonstrated his inability to participate in a group situation. In order to provide him with the level of support he required, a one-to-one situation was created for him. His main caregiver was his psychotherapist who worked with him in both play therapy and Sandplay Therapy. Through the therapist's analysis, it became apparent that Danny reacted in an extreme hyperactive mode as a result of his anxiety. In addition, observations showed that these phenomena would always occur in times of transition. A behavioral pattern emerged as a result: each time Danny faced a transitional situation, his anxiety level would rise. His inability to tolerate and cope with transition created a level of stress for him for which he emotionally "knew" only one way out - to explode!

In his therapeutic, one-to-one program, and in his more formal therapy sessions, Danny got in touch with his inner disintegration which occurred during each explosion. He also experienced the human container the therapist provided. He started to feel the "piecing together," the integration, that occurred with the aid of the therapist after each explosion. As a result, he was able to slowly start to develop his own boundaries. He also worked very hard to learn how to internally create for himself the support he required during these risky times of transition. Through Sandplay Therapy, he learned that inside of him there existed the capable hard working "agents" who can help him cope with his stress. During his play, within which he verbalized thoughts, feelings and wishes, Danny was able to recognize that he, as a human being, can be contained even when the lid is not on. He acknowledged that having boundaries also enables him to have the space within which nourishment and growth can occur. He expressed the realization that a transition can be tolerated and that he does not need to feel entrapped any more. As a result, he started to become aware of the fact that he, therefore, does not need to explode.

CONCLUSION

Through these illustrations one can realize how powerful the use of psychotherapy can be. With many of the above mentioned individuals, behavior modification was tried and failed. Psychotherapy was able to provide the children and adults who were illustrated above with the emotional support they needed. It also provided them with the opportunity to emotionally work through their difficulties. Play therapy, art therapy, drama, music and dance as well as Sandplay Therapy create an environment within which the person feels accepted and as a result is able to take the risk of facing him/herself (Baum, 1980). These therapeutic modalities and specifically Sandplay Therapy, also work on the unconscious level, thus, strengthening the evolving ego and empowering the emerging self. The patient learns to recognize his/her inner parts and become aware of his/her inner abilities.

This can create surprises such as the one that occurred during the 1992 graduation ceremonies at the Muki Baum Treatment Centre. One of the performances was supposed to be a song and dance about going to the ball game. The singer, a 12 year old girl with autism, experienced great difficulty and withdrew two days prior to the performance. Bob, an 11 year old boy, who started to attend the school only last year after experiencing great failure at his previous schools (he could not be contained and manifested this in extreme aggression) volunteered to sing. With some hesitation, his teachers agreed and he learned to sing "Take me to the Ball Game" in one day.

More than 200 people attended the graduation ceremonies. When his group's turn came, Bob stood up in front of the audience and in a beautiful clear voice sang the song twice. The applause was long and Bob, who started to attend the school being anxious and angry, stood there proudly accepting the audience's appreciation. At the end of the evening, Bob's mother came to me and said: "I am completely astonished. I never knew that he could sing." Bob's mother's words speak for themselves. With the aid of psychotherapy, someone like Bob can learn to cope with stress and anxiety in a different more appropriate manner. He can learn to appreciate and accept himself. He can open up and demonstrate the wonderful potential hidden within.

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TOWARDS DIFFERENTIAL REHABILITATION STRATEGIES IN EMPLOYMENT OF FRAGILE X ADULTS

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ABSTRACT

Eight fragile X and eight Down syndrome patients, all moderately mentally retarded, were tested with the Trainee Performance Scale, part of the Crawford Small Parts Dexterity Test and ranked with the Becker Work/Attitude Domain. Results show that fragile X patients have superior psychomotor skills and work habits. It was concluded that the main obstacle in the vocational rehabilitation of fragile X patients is their failure to focus attention on the given task. It was suggested that differential rehabilitation strategies for the fragile X person be developed to bypass the attention deficit disorders (ADD) shown by these patients.

INTRODUCTION

Retarded individuals are assessed by a multi-disciplinary team at the Jerusalem Elwyn (JE) assessment center. Assessment includes medical, psychological, social, educational and vocational evaluations. In the last decade, during which the professional community became more aware of the fra X syndrome, the staff of the assessment center began to notice that fra X adults with moderate mental retardation seem to show a characteristic vocational profile which differentiates them from mentally retarded individuals at the same level of retardation.

Nevertheless, examination of these patients' files and a sample survey of sheltered workshops in Israel reveal that staff at rehabilitation centers, professional and lay alike, disregard these characteristic features; and often, as a matter of fact, use the same rehabilitation methods and concepts for the fra X adults as for all other mentally retarded patients. These methods are widely known, and include: task analysis, modeling, rehearsal, kinesthetic assistance, facilitating devices and schedules of reinforcement. These methods certainly meet the needs of the retarded, especially the needs of Down syndrome patients.

Hagerman (1987), Largo and Schinzel (1985) and others have claimed that fra X patients are characterized by the presence of hyperactivity and short attention span. These characteristics have not been reported as being significant in Down syndrome (DS) patients who, on the contrary, showed greater social competence and less maladaptive behavior (Silverstein et al., 1985). Furthermore, the assessment center staff frequently reported that fra X examinees, after appropriate rapport has been established, display surprisingly good performance in vocational tests and seem to have a "built-in" repertoire of those skills needed to accomplish good results in simple technical tasks.

In this work, we show that fra X subjects have *a priori* enough psychomotor abilities to perform most of the tasks which are learned by DS individuals in sheltered workshops. It is also shown that, in order to bring the fra X subjects to a reasonable level of vocational performance, the trainer must bypass the obstacles imposed by the subject's attention deficit as follows:

1. Create a one-to-one setting with the client at the center;
2. Provide a relatively stimulus-free environment;
3. Continuously refocus the client's attention to the task, as soon as the client shows signs of distraction.

The present study amply attests to the fact that there is a need to look for new and different rehabilitation strategies to meet the specific needs of various groups of handicapped persons, such as the fra X patients. Differential methodology of rehabilitation must focus on the environmental conditions which minimize attention span and hyperactivity for the fra X person; while, for the DS patient, the classic strategies for training mentally retarded clients mentioned before, should be used.

METHOD

Subjects (Ss):

The Ss were sixteen moderately mentally retarded persons aged 20 to 32 years. Eight of the Ss belonged to the fra X group, and eight of them to the DS group. Age distribution in each group was similar. None of the Ss presented a history of progressive mental deterioration.

APPARATUS

All Ss in the two groups were given the Trainee Performance Sample (TPSD) (Irvin et al.) which measures the individual's ability to perform tasks which were broken down to their small components. In this test, three types of instruction -- verbal directions, model/match-to-sample, and physical prompts -- were given to Ss, and their level of performance for each type of instruction was measured. The task attributes represented in the test across all items are: (a) difficult discriminations; (b) coordinated two-hand movements; (c) sequence of steps; and (d) conjunctive stimulus control.

The Crawford Small Part Dexterity (SPD) Test (Crawford & Crawford, 1981) was administered to all Ss to measure fine eye-hand coordination and dexterity. The Crawford Test was also used to assess Ss' attention-to-task span. Part of the Becker Work Adjustment Profile (BWAP) (Becker, 1989), a five-point descriptive-graphic rating scale with a work habits/attitudes domain (HA) was used in their work to assess Ss' traits with regard to behavioral and attitudinal expressions.

SETTING

The TPS and Crawford Test were administered in a testing room at the JE assessment center. The room was silent; a table, two chairs and only a few other objects necessary for testing were in the room. Testers were experienced both in the use of the test and in working with disabled persons. They established a good rapport with Ss, made the Ss comfortable during the test, and reinforced the Ss profusely for their performance.

PROCEDURE

Ss were tested individually in one session. Ss were told they were taking part in research on the subject of vocational rehabilitation. Testers helped allay anxiety and fears of the Ss, due to any misunderstanding of the research. The self-esteem of all Ss was enhanced by the tester before testing began.

The TPS was first administered to each subject as specified in the administration manual of the test. Following administration of the TPS, the Crawford SPD was given to the Ss. The first task consisted of placing a screw in a threaded hole, twisting it until it started in the thread, and then turning it down all the way using a screwdriver. The number of trials used by each subject to master the task was registered. After the subject mastered the task, he was required to repeat it six times (1 row of 6 threaded holes). The Ss were told that they would be timed and were asked to work as fast as possible. The time was recorded for each subject.

In order to obtain a measure or an index of distractibility, or in other words, the subject's ability to concentrate on the assigned task, we recorded the number of times each subject was distracted from his task. With the tester's mediation, the subject's attention was immediately refocused on the task. Two kinds of "distractions" were recorded: inter-trial distractions, where the subject failed to continue performing after finishing a given trial and before becoming engaged in the next; and intra-trial distractions, where the subject failed to continue performing while engaged in a trial. Upon completion of this test, Ss were told that they had been of help, and were dismissed.

To obtain the ratings of the BWAP, either a case manager or one of the parents of each subject was interviewed by a trained member of the JE staff, who ensured that the interviewee knew the subject will enough to provide reliable answers.

RESULTS

Figure 1 is a graphic representation of the average rating obtained by the subjects of each of the groups separately on the five items measured in the BWAP. It can be seen that there is a tendency toward better rating in work habits within the fra X group than in the DS group.

Tables 1 and 2 show the scores obtained by the fra X group and DS groups, respectively in the TPS. Table 3 shows the mean and standard deviation of the TPS performance for both groups.

FIG. 1

BECKER'S WORK HABITS / ATTITUDES DOMAIN (HA)
(MEAN ± SD OF THE SCALE)

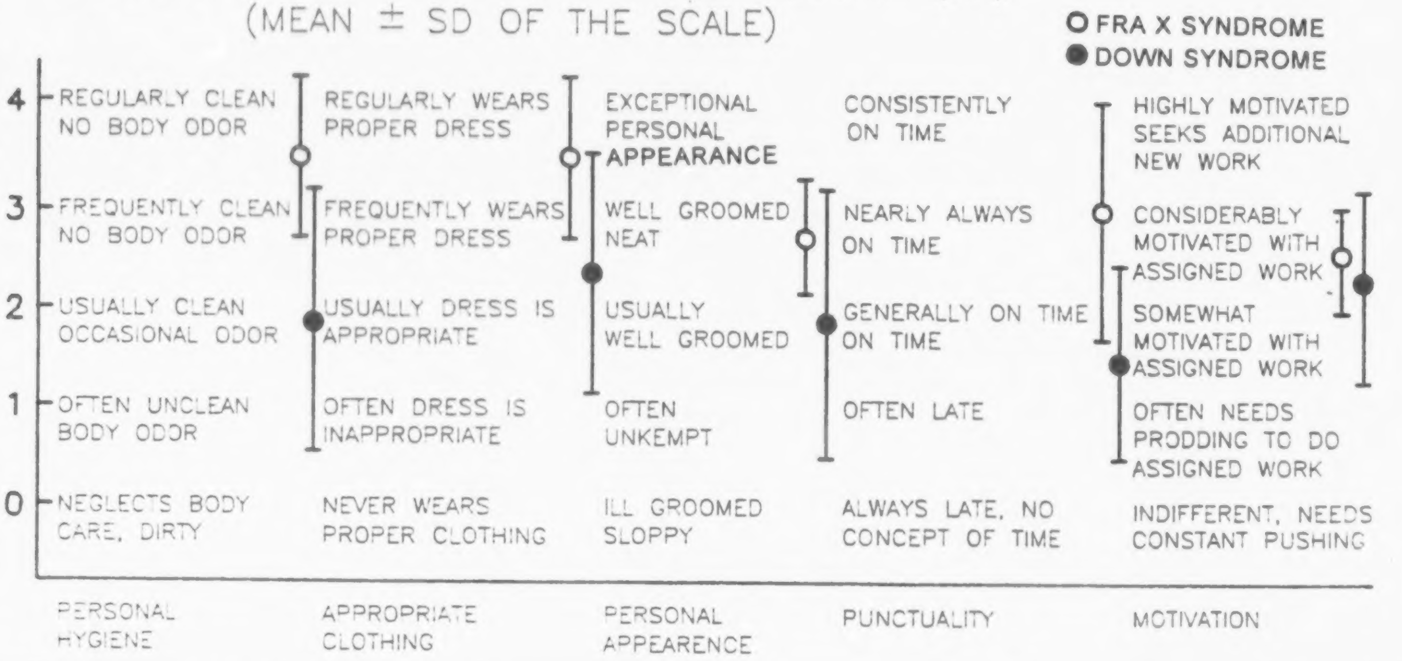

Table 1: Scores Obtained by the Fra X Group in TPS

PAT. NO.	AGE (YEARS)	T P S (%)		
		TOTAL	VERBAL	MODEL
1	30	88	71	100
2	28	86	79	83
3	20	90	82	65
4	23	78	86	65
5	32	98	100	100
6	20	96	82	100
7	27	82	57	94
8	22	84	71	83

Table 2: Scores Obtained by the Down Syndrome Group in TPS

PAT. NO.	AGE (YEARS)	T P S (%)		
		TOTAL	VERBAL	MODEL
9	29	54	21	78
10	24	52	21	51
11	31	66	43	61
12	29	52	14	67
13	21	56	54	56
14	24	46	21	56
15	20	56	50	44
16	32	68	50	67

Table 3: Mean Scores ± Standard Deviation For Each Group
Total TPS Scores

GROUP	N	MEAN (%) ± SD
FRA-X	8	87.7 ± 6.3
DOWN SYN.	8	56.2 ± 6.8 *

* $P < 0.001$

P VALUE IS DERIVED FROM STUDENT'S t TEST

The Crawford SPD Part 2, Screws, shows 1-3 learning trials with an average of 1.6 ± 0/7 for the fra X subjects; 3-7 learning trials with an average of 5 ± 1.7 for the DS group. The average performance time was 2'36" ± 1'36" and 4' ± 2'55 for the fra X and DS groups respectively. Inter- and intra-trial distractions were found in all fra X subjects except subject 1, who had no intra-trial distraction. None of the DS group had any inter- or intra-trial distraction.

DISCUSSION

Results seem to imply that the main problem to be solved in the vocational rehabilitation of fra X patients is their tendency to be distracted by what seems to be both exogenous and endogenous stimulation, in other words, their attention deficits. The average TPS score obtained by the fra X (87.7) shows that the Ss have well-developed overall skills with materials and instructions commonly used in sheltered workshops. On the other hand, the average TPS score for DS patients (55.2) shows that, although they have some skills, they require repeated practice on many tasks. The verbal score shows a significant advantage of the fra X group for verbal instruction.

The main problems were exemplified by the performance in the Crawford test: all the fra X Ss showed many inter- and intra-trial distractions, while none of the DS Ss showed distraction at all. On the other hand, fra X Ss learned the task immediately, while DS Ss required five trials on the average. In the test setting, ADD was almost neutralized by the tester's prompt intervention each time a subject seemed to be distracted from his assigned task. Under such conditions, the average performance time of the fra X Ss in the Crawford test was half that of the DS Ss. Unfortunately, we did not test fra X Ss' performance without tester intervention, but it is more than plausible that without intervention, the Ss would never have completed the tasks. Results of the Becker's Work Habits/Attitude Domain show that fra X Ss tend to obtain better ratings in the vocational adjustment variables.

In summary, basic work and vocational adjustment skills of the fra X Ss are good and, in the present study, these skills prove to be better in the fra X Ss than in DS Ss of similar age and level of mental retardation.

The rehabilitation of retarded adults in sheltered workshops, the most frequent placement for retarded adults, is based on enhancing the client's work performance skills and his vocational adjustment. Present results clearly call for a differential approach to the vocational rehabilitation of fra X clients. Individual assessment must include some evaluation of the degree of ADD symptoms shown by the client and of effective ways of dealing with the problem. Furthermore, the work environment must be engineered according to the need of the fra X subject: quieter rooms, fewer workers, and more staff. The counselors in the work room must refocus the client's attention to the task when necessary. Likewise, pharmacologic intervention to reduce ADD symptoms at work should be considered in further research.

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STAGES IN VOCATIONAL TRAINING FOR FRAGILE X CLIENTS

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During the last few years, there has been a change in attitudes regarding the developmentally disabled. As a result, increased emphasis has been placed on "independence," encouraging many members of the developmentally disabled community to live within our society as functioning human beings.

Three major goals of the rehabilitation process of the adult developmentally disabled are:

1. Development of social skills necessary for suitable behavior in society;
2. Vocational rehabilitation according to individual capabilities and placement within a protective framework or within the productive and competitive work force; and
3. Placement within a special hostel to function with maximum independence according to capabilities.

This paper will focus on the rehabilitation evaluation and placement of fragile X syndrome (fra X) clients in the productive work force. The results of our observations and diagnostic testing at the Jerusalem Elwyn Diagnostic Center and Sheltered Workshop demonstrate the superior manual motor abilities of the fra X clients.

We found that the variants: initiative, self expression, independence and relationships with colleagues at work, as well as perseverance, concentration and adaptation to the work place, received low grades during the course of the observation period. Other variants such as precision, quality and the understanding and memorization of given orders, received very high grades during the observation period. Restlessness and difficulties in concentration are well known characteristics of fra X and constitute the major difficulties in placing these people within any social framework, including employment. According to our evaluation, some of the factors related to this restlessness and lack of concentration include anxieties, lack of inter-personal communication and mistrust of the caretaker. We find that although patients with fra X show the ability to accomplish technical tasks, their difficulties in communication and in maintaining inter-personal relationships reduce productivity.

Our rehabilitation program, originally created for a mentally disabled client population, has been expanded in recent years to include people with a variety of limitations such as personality disturbances and psychiatric disorders. The goal of this program is successful placement of the rehabilitated client in the work force.

The program as adapted to the fra X clients, builds upon the following:

Stage A:

Getting to know and acquiring a trust of the client, his family or the direct caretaker by holding personal meetings in a familiar environment.

Stage B: Evaluation and early training.

At this stage, the candidate joins a training group and works in a productive plant in the open market for a period of approximately six months. At the end of this period, the team prepares a recommendation regarding a permanent future placement.

The training group consists of three clients and an instructor. We have set up five main goals to be accomplished within this framework.

1. Reduce anxiety stemming from the very concept of work;
2. Ensure that the candidate has a positive experience for the first time in a regular job;
3. Gradual adaptation to an eight hour work day;
4. Acquire work habits; and
5. Acquire concepts and social habits acceptable in the working world.

Stage C:

When the candidate can perform all tasks required in Stage B, we will try to find suitable employment. The instructor goes to the potential employer and spends several days in order to study the work and prepare a task analysis. When the instructor is ready and familiar with the various tasks, the candidate will join the instructor and learn the respective tasks without stress or demand for a certain output rate. When the candidate attains a certain level in his performance, the instructor will accompany the candidate less frequently. The instructor will nevertheless be around the production line within the plant and be available to the candidate. At a later stage, the instructor may also leave the premises for several hours and return at set times, gradually lengthening the absence periods.

When the candidate reaches a satisfactory level of performance, is comfortable in the work place, and satisfies the employer, monitoring is reduced to an hour per day and supervision gradually reduced to random visits on different days until there is just one visit per month.

Stage D:

Follow up includes occasional meetings with the client, his family and the employer as necessary. The follow up is administered and supervised by the case manager.

From our own experience, we have found that this stage is an integral part important to both candidate and employer, and inevitably guarantees that the candidate will be accepted on a permanent basis at his job. This particular stage is vital in the process.

The fact that we remain involved facilitates supportive interaction in all cases of difficulties between worker and employer, and provides the client with a sense of security and dependable support.

TREATMENT STRATEGIES FOR ADOLESCENTS AND ADULTS WITH FRAGILE X SYNDROME

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As children affected with fragile X syndrome grow and mature, a new list of challenges and opportunities awaits these young people and the people around them. These include family, healthcare professionals, the educational community, and a multitude of other professionals responsible for vocational training. This paper will provide a brief discussion of some of the more salient points for consideration in effecting an optimal outcome as children approach adolescence and adulthood.

EDUCATION IN LEAST RESTRICTIVE ENVIRONMENT

Leaders in the educational community strongly believe special education students, including those with the most severe disabilities, learn best in natural, diverse and integrated school and community environments. A recent study conducted by the Educational Testing Service examined integrated programs for students with learning disabilities at 14 school districts in 9 states. The study concluded that integration had *strong positive results*. It is important to be cognizant that most fragile X children's performance will be higher than IQ scores indicate.

It is widely recognized that patients affected by fragile X have outstanding mimicking skills. Placing these young people in classes with normal children will provide benefit to both parties. Those closest to the fragile X persons must be very perceptive to the hypersensitivity that fragile X people have to their environment, and be responsive to the demands made upon these young people..."Know when to back off and redirect."

Classroom sizes are small and afford students as much individualized curriculum as possible. *Continuum of a multi-disciplinary concept for education will provide a greater chance to achieve a more desirable and optimal outcome.* For students whose IQ is below 70, better results are achieved using a more "vocational setting approach."

The vocational curriculum prepares the young person approaching adulthood "for preparation into adulthood as active participants in an integrated society." Critical objectives are achieved in areas of:

1. Integration - emphasizing commonality in all students
2. Curriculum - providing students with daily instruction in domestic, community, leisure and vocational environments
3. Transition - from school into integrated adult environments. Transition planning is initiated early in secondary school years which fosters optimal success in

adulthood. Continued vocational support in adulthood should be established before graduation. *Jobs offering competitive wages in integrated settings are the ultimate goal.*

DEVELOP AND NURTURE AREAS OF STRENGTH

When areas of strength are identified by family, the medical community and the educational community for a fragile X patient/student, every attempt should be made to nurture these strengths. Specific examples might include the following:

Music

Provide opportunity to play an instrument if possible. Music teachers and music therapists have found that fragile X patients/students enjoy particularly the low brass instruments that have an unique tone/sound. Many are able to play by ear and learning to read music is achieved in many cases.

Travel

It is widely recognized that people affected with fragile X are frequently **visual** learners and also retain well when associated with life experiences.

Computers

Computer use emphasizes visual learning strengths. A great number of software programs are available and on the market. {Note: Facilitative communication is another excellent tool to explore on an individual basis.} (See McClennen and Shopmeyer in these proceedings.)

HORMONAL/PHYSIOLOGIC CHANGES WHICH MAY AFFECT MEDICATION

As children affected by fragile X mature, physiologic changes occur which affect behavior, and the need for medication changes. Hormonal changes occur as with any adolescent which may prompt the need for adjustments in dosing or a change in medication. Stimulant drugs may lose effectiveness. Anxiety and aggressive behavior, particularly in males may occur. If signs of anxiety and/or aggression appear, caregivers must be alert and respond to these changes and refer the patient to his physician for appropriate treatment.

ADULT LIFE

When adulthood is achieved, the family and caregivers of persons with fragile X must assist these young people in making the choices right for them. These include whether to live independently; in a group home; in an apartment with one or two others with supervision; to remain at home; or choose other alternatives. The person's ability to function independently will weigh heavily in the decision making process.

The long range benefit from integration is that these young people will be looked upon as ***real people who share the work with us***. Given the benefit of early diagnosis and aggressive early intervention, many of these young people have the capability to lead a productive and fulfilling life that in return, can warm the hearts of those caregivers that have come to know these special individuals..."The most rewarding things you do in life are often the ones that look like they cannot be done."

DEALING WITH THE DIAGNOSIS

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Tom Olson and Bonnie Sunstein, PhD

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The panel consisted of five parents, four mothers and one father, with children affected by fragile X syndrome. The children ranged in age from 8 years to their mid-twenties. The panel began by sharing their personal experiences of receiving the news of the diagnosis.

Tom began by relating his experience of shock, confusion and disbelief in learning of the diagnosis the day after his youngest (unaffected) daughter was born. He received little positive information at the time, being told that fragile X had been found in severely retarded males. He began a pilgrimage through the medical journals seeking hope and positive information, finding instead mostly highly technical genetic research and population studies. Through the years, he came to a gradual acceptance and positive outlook on a son who is now perceived as a remarkable gift from God.

Mary Ann learned of the diagnosis shortly after Nathan's birth and an 8 1/2 month period filled with grief at losing both her parents and her husband's parents. With the help of a perceptive pediatrician and referrals to Dr. Hagerman in Denver, she received needed positive information and found fragile X to be a place to put her energy and to work through some of her grief.

Bonnie Sunstein, mother of an affected son and affected daughter, described her experience as a fragile X parent as falling into three stages. Having learned of the diagnosis when her children were 10 and 13, she described the early years as the "babyhood and primary schooling stage" in which there was a complete lack of knowledge about fragile X syndrome. The second "schooling-intensive" stage was characterized by years of trying to balance the special education services and education plans in which her children endured label after label after label. The third stage she characterized as the "relief" stage in which, after receiving the diagnosis, she finally had an accurate "label" which could be used as a tool for facilitating proper educational services.

Sandy Brooks related her experience of intuitively knowing something was genetically abnormal about her son after birth. She knew this from the perspective of having worked as a nurse in the field of labor and delivery. Her son was diagnosed by a personal visit from Dr. Hagerman as she was presenting at a nearby conference. Though diagnosed at age 7, her son had received excellent special education and therapies up until and after the diagnosis was made.

Shannon Geis, mother of both a daughter and son with fragile X, received the news in the mail as a "birthday present." Her grief about Eric had passed by the time he was four years old, but now she had to deal with the shock of having something "wrong" with her, being labeled a genetic carrier. Her family was less than supportive, especially about being genetically tested.

The panel discussion centered on the strengths of the fragile X child. Mary Ann shared the often overlooked positive outlook on life radiated by fragile X children. Her son Nathan displays a "love of life," a love for others and a positive outlook despite external circumstances to the contrary. Bonnie shared her teenage daughter's strength in imitating, especially heavy metal bands and other friends of the teen subculture. Sandy described her numerous positive experiences with son Craig in travelling throughout the world in which Craig naturally imitates the accent of the country.

Sandy shared her positive experience with son Craig in utilizing facilitative communication. She defined facilitative communication as a force or resistance to a person in pointing, spelling, or typing on a communication board, allowing that person to create language on his own. She stressed that the technique cannot hurt a person and may facilitate language for those previously defined as non-verbal. She related that Canon computers designed for this purpose may be available through Canon or Easter Seals at minimal cost.

Regarding communication in general, Bonnie commented on the problem of our society's definition of "literacy" as verbal fluency. An interruption of fluency does not equate with an inability to communicate, she explained. Shannon focused on the need for fragile X parents to truly *listen* to what their children are attempting to say, to be extremely cautious in speaking on behalf of them due to their problem in communication. Mary Ann stressed the importance of enabling our children to express their *feelings* openly, even though the words may be truncated or seemingly inappropriate at the time and place.

Medical concerns were discussed from the perspective of the family. Fragile X children frequently do not experience pain the same way as other children. Tom shared his son's experience of having caught his finger in the door of an automobile as it closed and the significant delay of several seconds before Matthew expressed the pain. He related the experience of another affected child who nearly died from appendicitis because the surgeon did not know the child did not experience the customary rebound tenderness indicative of an acute attack. Sandy emphasized the need for parents to be extremely perceptive of their children's expressed discomforts as a problem may be amplified many times over the normal child's complaints.

The panel discussed the importance of good nutrition for fragile X children. Bonnie related that many fra X children "perseverate in eating" a particular food just as they perseverate in speech. Tom shared that the most difficult behavior problem with his son was at dinnertime, whether it be due to the sensory overload of smell, sound, texture of the food, or a combination of all three. Bonnie related the humorous experience of asking her son, "How many apples did you eat?" after school. "Just one, Mom." After a time she caught on and asked him, "How many times did you have one apple?" She discovered that his view of time and quantity was not the same as hers and this needed to be considered in family nutrition.

The panel discussed ways in which they deal with the emotional stresses within the family that affect the fragile X child. Mary Ann described her basement "gymnasium" of sensory integration equipment that has helped Nathan. Shannon related her positive experiences in utilizing sensory integration techniques with her son who is in his 20's. Providing routine, structure and planning well in advance were emphasized as positive ways to help fragile X children cope with emotional stress. Placing information on calendars such as smiling faces on dates of special occasions may prove helpful with some children.

Children with fragile X may face some unique identity issues during their adolescence. Shannon described the pain of answering her son's question about the age of 10. "Why am I different, Mom?" Initially, she explained that we all are "different," having different hair, eyes, skin, etc. and that it is important to be different. This answer was sufficient for a few years until her son rephrased the question. "What does it mean when they say 'that boy is retarded', Mom?", he asked. She fielded this question by reviewing his early life and explaining how he was different in his way of learning and emphasizing that it was okay to be different.

The panel discussed the importance of just having fun together with their fragile X children. This may take the form of playing ball together, even though the child may catch only one in a hundred. But one panel member stressed that the needs of the child must be realistically taken into account when determining what fun is. For example, it took several years for one parent to realize that attending firework displays on the Fourth of July may be fun for the parent, but uncomfortable for the child.

There was a discussion among panel members of the generalized resistance of extended family members to be genetically tested. The extended family often provides a minimal amount of emotional support to newly diagnosed families. Mary Ann and Shannon both described their own frustration in providing the information to their families and not finding much interest or support. Mary Ann explained that she has grown to perceive her family as more than just her biological family. Many fragile X families have become as close to her as her biological family.

Sibling relationships were discussed within fragile X families. Tom shared three different children's experiences in dealing with his affected son, including maternalism, embarrassment, competition, compassion and pity. Bonnie's teenage children show an extreme sensitivity and loyalty to each other, never fighting with each other. Mary Ann's youngest son plays well with her affected son, Nathan, and they have a very positive relationship together.

Family planning was addressed by Mary Ann as an *avant garde* issue facing newly diagnosed families. She personally faced much criticism for wanting to have another (possibly affected) child as well as for *not* wanting to have another child. She stressed the importance of the need for each family to feel the freedom to decide either way and follow their conscience. She challenged the implied social sanction that "just because I have a genetic condition I should not have another child."

The group closed by centering on the positive effects of raising a fragile X child. The panel concurred that parenting a fragile X child changes one by challenging beliefs, values and relationships. The concept of growth and "personal maturity" was used to describe this process. In some cases, marriages were strengthened, in others, marriages did not survive. In some cases, friendships did not survive and new friendships were forged based upon a sifting of values and strengthening of commitments to what was deemed truly important in life.

Dealing With the Diagnosis

The panel proposed the following list of books as resources for families seeking help in dealing with the diagnosis:

Dorris, Michael. *The Broken Cord.*
Lee, Christopher and Jackson, Rosemary. *Faking It.*
Nouwen, Henri J. *The Road to Daybreak.*
Simon, R. *After the Tears.*
Stires, Susan. *With Promise.*

FACILITATING A FRAGILE X PARENT/FAMILY SUPPORT GROUP

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The objectives of this workshop were to prioritize the responsibilities of a support group and to discuss methods for fragile X families to integrate with other special needs families who have the same general concerns for their children, i.e., educational programs, socialization issues, etc.

There are several models of support groups: 1) parents; 2) families; and 3) families and professionals. In most cases the families and professionals groups tend to be the most constructive. For example, the Fragile X Association of Washington is a family group which meets every other month for support and sharing of concerns and ideas. On the off months, the families meet with professionals from the Child Development Mental Retardation Center at the University of Washington. This bi-monthly meeting usually has a guest speaker and a specific agenda. The alternating meetings allow for a balance to meet the needs of all involved.

Many family support groups start with the best intentions to meet the needs of the initial families involved. Often these groups can establish connections with one another, but if a new member joins, there is difficulty in integrating the new family into the original group. Maintaining a structure and allowing professionals to be involved can assist greatly in the continuity and effectiveness of the group, keep the group updated on new scientific and treatment information and also provide expertise to educate the community.

Another area for concern is the age of the special needs child/adult. Families with toddlers have very different problems and concerns than families with adult children. One strategy to consider is having two or three small groups, where the age of the affected individual determines with which group the family meets regularly. This might be a bi-monthly meeting with the alternate month for all the families and professionals involved.

REASONS FOR A SUPPORT GROUP:

- To allow families with common diagnosis to meet and share ideas
- To help families understand the special needs of their child and how best to meet those needs
- To share knowledge regarding community resources to enhance their child's development
- To receive needed support and knowledge from professional caregivers and other families
- To assist families in moving beyond initial reaction after receiving a diagnosis

- To encourage opportunities for the families to meet in an atmosphere that encourages deep and personal sharing of concerns

AREAS OF ASSISTANCE PROVIDED BY PARENT SUPPORT GROUPS:

- Support groups can provide emotional and psychological support on a level that is most beneficial to the families in need.
- Having a child with special needs can limit the opportunities for families to socialize. Support groups can provide a caring atmosphere in which the children can interact with one another without the risk of judgment by other non-supportive groups.
- The networking within a support group can provide valuable information about community resources, educational opportunities, legal rights, etc. to newly diagnosed families.
- The support group as a whole can serve as activists and advocates on behalf of special needs children.
- When families and professionals work together, they can improve community attitudes toward children with special needs.

TO START A FAMILY SUPPORT GROUP:

- A needs assessment should be conducted to:
  - define functions such a group might perform
  - determine topics of interest for informational meetings
  - decide the ideal group size
  - suggest meeting times and places-maybe not someone's house so everyone has chance for "time-out"

- Logistics to consider when creating a support group:
  - type of group, pros and cons of each
    - family
    - family and professionals

- Suggestions for committees to create within the group:
  - steering-for leadership until officers elected
  - research-maintain reference file on pertinent articles and resources
  - counseling-to assist newly diagnosed families
  - public relations-to inform the community about group and purposes: announcements of meetings placed in local newspapers, members of organization invited to appear on television or speak on radio about group

PROVIDING SUPPORT FOR NEWLY DIAGNOSED FAMILIES:

- develop information packets
- have members available for visiting
- develop and implement training for visiting families
- maintain contact with hospitals to share importance of group and early parent-to-parent support
- include siblings and extended family members in scope (contact the Sibling Support Project - Don Meyer, Director (206)368-4911, for ideas)
- teach principle of normalization-A special needs person will learn and develop in optimal fashion if he/she is treated normally and is included in those activities appropriate for his/her age group

ADDITIONAL IDEAS:

- consider providing childcare during group meetings-this can help families attend on consistent basis
- develop a library of pertinent information
- also plan purely social functions throughout the year

ALLIANCES WITH OTHER ORGANIZATIONS

This can also provide a more cohesive unit in dealing with issues and problems, also allying with the following types of groups can provide more authenticity to your group

- National -- For example, The National Fragile X Foundation has a Resource Center network which assists families, provides information and referrals and support to local groups
- Local -- organizations for other disorders with same general concerns

HOW TO MEET NEEDS OF ALL AGES WITHIN YOUR SUPPORT GROUP:

- Families with affected members of different ages have different concerns educational, vocational, residential, social, etc Try to create different structures to accommodate all ages

IDEAS FOR MEETING THE NEEDS OF FAMILIES:

- Provide follow-up by social worker, genetic counselor and previously diagnosed families, between diagnosis and acceptance Newly diagnosed families are suddenly faced with denial, anger, and many questions once they have had time to grasp the impact of a fragile X diagnosis. When a diagnosis is first received, many families need the opportunity to ask questions several days or even weeks later, when they have had time to digest the information. Providing follow-up from professionals and other families allows the newly diagnosed families an opportunity to get more information from a caring source.

- Create a buddy system, matching newly diagnosed families with families previously diagnosed (1-2 years prior) who have the same aged affected individual. This can be very helpful to both families, providing support and information to the newly diagnosed family and allow the previously diagnosed family to assist in areas in which they have become experts.
- Have information cards available about your support group for pediatricians, child development units, etc., to give to newly diagnosed families so they are immediately aware of your group.
- Provide professionals; educators, clinicians, special education therapists, etc, with a simple booklet explaining fragile X syndrome.
- Create a positive approach in working with professionals and families.
- Tie in with local service organizations, clubs, etc. The Rotary, Lions and others are all good sources of community involvement and they might provide a place to meet, refreshments, scholarships to conferences, etc.

Family support groups can be very beneficial to all involved. One item to keep in mind is to have several families and professionals participate with the organizing of the group. This will help to balance the group and keep one person from getting overwhelmed with the planning, etc.

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III. Diagnostic Testing: Cytogenetic & Molecular

THE MOLECULAR GENETICS OF THE FRAGILE X SYNDROME

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INTRODUCTION

The gene responsible for the fragile X syndrome was discovered in May of 1991 and this finding, along with others regarding the nature of the mutation, has resulted in a tremendous increase in our understanding of this very frequent genetic disorder. The FMR-1 gene (fragile X mental retardation-1) is approximately 70 kb in size and is expressed in both brain and testes as well as some other organs. The predicted amino acid sequence of the FMR-1 protein reveals this to be a unique protein, dissimilar from any protein or gene previously studied. Hence, it remains unclear what is the normal function of the protein and why its absence results in fragile X syndrome, although tremendous research efforts are focused upon these questions.

THE FMR-1 GENE

Within the FMR-1 transcript is a lengthy repeated DNA of the triplet CGG. Among normal individuals, this repeat varies between 6 and 58 repeats and usually is inherited stably from parent to offspring. In fragile X syndrome, this repeat undergoes massive expansion beyond 230 repeats, sometimes exceeding 1,000 triplets. Among unaffected male carriers (transmitting males) and most female carriers, the repeat is found to be of intermediate length between 52 and 200 repeats. Unlike the normal X chromosome, the repeat sequences on the fragile X chromosome are markedly unstable and are inherited from parent to child in a different size, usually larger in number. Strikingly, siblings who inherit this chromosome usually are genetically distinct, in terms of repeat length, from each other as well as from their parents. When this intermediate length mutation is passed on from the mother, it has a chance of undergoing the massive expansion to the "full mutation" resulting in fragile X syndrome in males. This form of expansion occurs only when transmitted from mother and not from the father. A transmitting male passes his mutation to all his daughters in a slightly different size, usually with minor increases. The repeat length in mothers does influence the chance of undergoing expansion to the full mutation such that mothers with 70 or so repeats have approximately an 8% risk of having an affected male while mothers with repeats exceeding 100 have a 50% risk of a male child with fragile X syndrome. The maternal repeat size similarly influences the risk of an affected daughter. However, the risk is greatly reduced since the normal X chromosome appears to provide a protective effect, such that only about 52% of females with the full mutation exhibit mental deficiency.

EFFECT OF THE MUTATION

Concomitant with expansion to the full mutation is an abnormal methylation of the FMR-1 gene. Surrounding the CGG repeat are sites for methylation-sensitive restriction endonucleases which cleave unmethylated DNA. Using these enzymes (chiefly BssHII and EagI), it can be demonstrated that these sites are unmethylated in normal and transmitting males while methylated in affected males. These sites are also methylated on the inactive X chromosome of females, as are numerous other such sites along the inactive X. The abnormal methylation of this region in affected males leads to transcriptional silencing of the FMR-1 in the great majority of fragile X males. The absence of mRNA will lead to the absence of the FMR-1 encoded protein, the deficiency of which presumably is responsible for the phenotype associated with fragile X syndrome. Since the CGG-repeat appears noncoding, as upstream in-frame stop codons have been identified as well as a downstream AUG-consensus sequence, the normal variation in repeat size and the expansion to the carrier state should not influence the protein structure or function. Only when the repeat length exceeds a certain size (approximately 230 repeats) does methylation occur with subsequent inactivation of the FMR-1 gene. Some affected males exhibit a complex pattern of methylation and repeat size variation in peripheral blood. These males are termed mosaic males to reflect the variation and these males frequently display evidence of FMR-1 gene expression. The expression is likely due to some cells which have reduced methylation, perhaps in reflection of a shorter repeat length, allowing expression of the gene. It is quite unclear if the protein produced in these patients and, thus far, there is no consistent evidence suggesting that these males are less severely affected.

MOSAICISM

Silencing of the FMR-1 gene on the inactive X chromosome also tends to occur based upon methylation studies and likely accounts for the lowered incidence of mental retardation in females. Normally, the choice of which of the two X chromosomes in a female cell becomes inactivated is random, such that females are "mosaics" composed of two populations of cells distinguished by which X chromosome is active. Since gene products are produced from the active X, this random inactivation is of little consequence. However, if one of the X chromosomes has a fully expanded CGG-repeat with the associated methylation at the FMR-1 locus, different outcomes are possible. If the fragile X is the inactive X chromosome, that cell (and its cellular progeny) will still produce FMR-1 gene product from the active, normal X chromosome. However, if the fragile X is the active X, no FMR-1 gene product will be produced since the abnormal methylation has silenced the gene on the active X and normal X-inactivation has silenced the FMR-1 gene on the normal, but inactivated, X chromosome. The frequency with which each cell type occurs in the body, and importantly, in relevant cells or tissues (i.e., brain), likely influences the occurrence and degree of mental deficiency in females with the full mutation. Thus, some females are protected, since the relevant cells produce sufficient FMR-1 gene product from the normal, active X chromosome. Currently, there is no way of predicting in females with the full mutation whether or not the individual will be affected. Based upon empiric data, the risk appears to be approximately 52%.

DIAGNOSIS AND PROGRESSION

Laboratory diagnosis of the fragile X syndrome has been greatly facilitated by this molecular understanding of the mutation. Both the length of the CGG-repeat and the methylation status of the FMR-1 gene can be readily determined from peripheral blood and from samples obtained for prenatal diagnosis. Greater than 95% of patients with a strong clinical suspicion of fragile X syndrome (i.e., X-linked MR with macroorchidism) exhibit expansion of the CGG-repeat beyond 230 triplets with methylation of the FMR-1 gene. Thus far, all mothers of such males display either the carrier expansion or full expansion of the CGG-repeat. No new mutations have yet been observed and screening for carrier status of relatives is therefore indicated.

EXCEPTIONS

A minority of patients, less than 5%, display mutations other than this typical form. Two patients have been described with deletions of the FMR-1 gene. These patients exhibit typical facies, macroorchidism and mental retardation. However, they do not exhibit the fragile X site on chromosome analysis and thus far, these cases have been sporadic. These patients presumably exhibit the Martin-Bell phenotype (without fragile X expression) because of the lack of FMR-1 gene product due to the deletion (in the two patients reported thus far, the deletions are large enough to also have deleted other nearby genes, yet undiscovered). Much effort is also underway attempting to identify patients who have point mutations in the FMR-1 gene who would be expected to show the fragile X phenotype (perhaps to varying degrees) without fragile site expression.

A NEW FRAGILE SITE

Several families have been found in which the diagnosis of fragile X syndrome had been made based upon familial mental retardation and expression of a fragile site on the distal portion of the X chromosome long arm. These patients do not, however, exhibit any discernible alteration in the FMR-1 gene. The repeat length and methylation status is normal and there are no obvious structural changes in the FMR-1 gene. These patients have been found to have another fragile site (designated FRAXE), distal to the fragile X syndrome site (FRAXA). Under routine cytogenetic examination, this fragile site is indistinguishable from the FRAXA site. However, through examination by fluorescent in situ hybridization, this site becomes distinct from the fragile X syndrome locus. Therefore, these patients do not have fragile X syndrome or the Martin-Bell phenotype and the exact nature of their mental retardation is unclear since it remains unknown if the FRAXE site is associated with the clinical problems in these patients or if they have been linked by ascertainment biases. Finally, it is important to note that cytogenetic examination should be performed on all patients where one is seeking to rule out fragile X syndrome since other structural or numerical abnormalities in the chromosomes can lead to mental retardation.

FUTURE DIRECTIONS

There are several very exciting avenues for new research in fragile X syndrome. These include such basic studies as understanding the basis of the fragile X mutations and the mechanism(s) that contribute to their unstable character. These studies will be of great significance to other genetic disorders involving similar repetitive sequences and to fundamental issues of DNA replication and repair.

Of greater significance to affected individuals will be the ongoing analysis of the FMR-1 gene and its protein product. Once the normal function of this protein can be identified, methods of treating fragile X syndrome previously unconsidered may present themselves. Knowledge of the affected tissues and the type of pathology will likely alter our view of the disease and the course of treatment. While unlikely in the near future, gene replacement therapy may represent an option as well. Tremendous progress has been made in the past year; it is likely that equally significant advances will be seen in the coming years.

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THE FRAGILE X SYNDROME: CURRENT UNDERSTANDING OF THE NATURE OF THE GENETIC LESION CAUSING THIS DISORDER

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THE PROBLEM

Inherited forms of mental retardation (MR) that result from defects on the X chromosome affect at least 1/500 males and leave about 1/400 females at high risk for being carriers. As there is presently no cure for these disorders, prevention of these high frequency, high burden disorders can best be achieved by accurate carrier assessment and genetic counseling. The major problem is identifying carriers. With the discovery that a subgroup of individuals with X-linked MR have a fragile site on their X-chromosome (the fragile X syndrome), a means for mapping this disorder with the eventual aim of identifying the culprit gene became apparent. Last year this gene was cloned (and termed the fragile X mental retardation gene-1: FMR-1) and this led to the establishment of a means for carrier assessment that is not risk-based, but which is virtually absolute: males and females are carriers or non-carriers based on some simple molecular tests.

The purpose of this presentation is to describe:

1) X-linked mental retardation and the importance of clinical information
2) Carrier detection
3) The fragile X syndrome
    a) Cytogenetics
    b) Clinical information
    c) DNA studies: Linked DNA markers
    d) DNA studies: FMR-1 gene
4) Significance of new tests

X-LINKED MENTAL RETARDATION (XLMR)

About 2.3% of the general population in industrialized countries have some degree of developmental disability. Although the causes are numerous, several forms have a genetic basis. It has long been recognized that there is an excess of males within the MR population (Penrose, 1938), most of which can be accounted for by X-linked mutations (reviewed by Opitz, 1986). In 1943, Martin and Bell published the first pedigree clearly demonstrating an X-linked recessive form of MR (XLMR). Since then, there have been many families identified on the basis of a prevalence of retarded males and probable transmission through females. In some cases, such as Hunter syndrome and Lesch-Nyhan syndrome, the biochemical defect is known.

XLMR comprises a very heterogeneous group of disorders with a carrier frequency of about 2.44 per 1000 females. To determine whether the same form of XLMR is present in two or more unrelated families, there must be a distinguishing phenotype (appearance), i.e., some characteristic which is found in all males with the same mutation. Of the non-specific forms of XLMR, the fragile X syndrome (fra X) is best studied since families with this syndrome can be identified on the basis of a specific chromosomal abnormality. In other cases, families can be grouped because of their similar facial appearances and other specific abnormalities. A large group of families have, however, been classified simply on the basis of a non-specific phenotype. The latter undoubtedly represent mutations in several different genes, with 49 being identified to date (Neri et al., 1991).

CARRIER DETECTION

The importance of carrier detection in the counseling of families with XLMR is well-recognized. Women at risk for carrying the disease allele have various options. Among these are different forms of contraception, including sterilization. Alternatively, decisions to undergo therapeutic abortion may be considered with prenatal diagnosis of the sex or, when possible, prenatal diagnosis to determine affected/unaffected status of the fetus. DNA marker or gene testing has been developed for several disorders. We and others have been using this approach to develop a means for carrier assessment in the fragile X syndrome.

THE FRAGILE X SYNDROME

Cytogenetics

The fragile X syndrome is the most common inherited cause of MR in males, accounting for at least one-third of XLMR and affecting 1/600 to 1/1000 males in the general population. It is an unusual X-linked disorder because about 20% of the males are unaffected, or very mildly affected, and about 30% of heterozygous females show some degree of mental impairment. Males with fra X have a characteristic phenotype, including large ears, distinctive facies, and macroorchidism (Hagerman, 1991).

Distinction between fra X and other forms of XLMR is based on the identification of the fragile site, which is a break at the tip of the X chromosome in cultured cells (e.g., lymphocytes, fibroblasts, amniocytes or bone marrow cells). This was first described in 1969 by Lubs. A special medium is needed in order for this abnormality to be detected in the cytogenetics laboratory. However, this requirement was not immediately recognized and it was not until 1979, when Sutherland described the importance of special culture conditions, that other investigators started to find additional cases of the "marker-X" or fragile X syndrome.

The cytogenetic test was an important step: now there was a biological "marker" which distinguished one of the forms of XLMR from all other forms. As we shall see, though, there were complications:

a) There was great variation in the percentage of fragile X cells seen for each patient studied (i.e., 2-60% of the cells with the broken X chromosome),

b) The percentage of cells with the fragile X was lower in females and was often not found in many of the mothers of fragile X boys,

c) Some families had males who apparently transmitted the fragile X mutation to their daughters. These males had both normal chromosome findings and normal appearance.

As a result of these difficulties, several investigators were developing different methods for inducing expression of the fragile X. unfortunately, although some methods were much better than the original media used by Lubs and Sutherland, the problems described above remained.

Clinical Information

Once methods were established for detecting the chromosome abnormality, it was possible to re-examine the affected males with this lesion for common clinical findings. The most striking features were found to be large ears (often protruding), long face, large jaws (particularly in the older males), and large testes (macroorchidism). Thus, instead of the fragile X syndrome having a "non-specific" phenotype, suddenly it was apparent that many of the affected individuals could be identified by these relatively mild clinical signs. It should be remembered, however, that many unaffected individuals have large ears or any of the other characteristics and do not have the fragile X syndrome.

Mental impairment can be relatively mild to severe. There may be other neurological, behavioral and psychiatric problems (including seizures, attention deficit disorder, hyperactivity, aggressive outbursts, and autistic-like features). Other physical abnormalities include ophthalmologic and orthopedic problems, unusual growth patterns, high arched palate, etc.

Of greatest importance is that this sub-group of developmentally disabled males can be recognized somewhat reliably from photographs, so that when a couple are concerned about "mental retardation in the family," photographs of the affected individuals can be helpful in determining whether this is a possible diagnosis. Confirmation requires either chromosome or DNA testing as outlined below.

DNA Studies: Linked DNA markers

In 1981, we initiated a study aimed at isolating and characterizing DNA markers which were closely linked to the fragile X syndrome locus. At that time, Drs. Dushan Soudek, Michael Partington, the Developmental Counselling Service at Ongwanada in Kingston, Ontario, and I had identified fra X positive individuals in 2 large and several small families and we had begun screening for additional families. We now are studying 170 affected males from 77 families with this syndrome. Our first DNA marker studies on the fragile X syndrome were reported in 1983, at the First International Workshop on the Fragile X and XLMR (Holden et al., 1984). At that time, we were the only group doing DNA studies on this disorder. Since then, the widespread nature of fra X and its prevalence have prompted many investigators to embark on collaborative studies aimed at developing panels of DNA markers suitable for linkage studies and a consortium of investigators from all around the world was formed in 1989 to develop good carrier testing methods.

The principle of using linked DNA markers is as follows. Chromosomes are made up of DNA, with long stretches of DNA forming each of the many genes along a chromosome. Genes are the basic units of heredity and they specify all functions necessary for cells to survive, as well as all of our characteristics. When a gene is mutated, it may cause a disease. For example, we have genes which produce proteins which allow us to see different colors. When one of these genes is mutated, the person is color-blind. Many genes are important in muscle function; however, when a specific muscle protein is defective, the individual has muscular dystrophy. Several genes are important for mental functioning: when the FMR-1 (fragile X mental retardation-1) gene is defective or missing, that person has the fragile X syndrome.

In between genes, there are "spacers" and other DNA sequences which do not seem to be important in performing any particular cell functions. Defects or differences in these regions of DNA are not clinically significant and so are tolerated without much consequence to the individual. We use those differences (called RFLPs or DNA markers) to trace defective (mutant) genes in a family. This is illustrated in Figure 1.

The carrier female (I-2) has a fragile X chromosome and a normal X chromosome. Her partner (I-1) has a normal X chromosome and a Y chromosome. There are differences in the DNA sequences along the X chromosome in both individuals, such that at "Locus A," I-1 has variant (allele) A1, and I-2 has alleles A2 and A3. On the other side of the FMR-1 locus is "Locus B": I-1 has allele B1, I-2 has alleles B2 and B3. Son II-1 receives the A2B2 chromosome from his mother and is normal. Son II-2 receives the A3B3 chromosome from his mother and is affected. The daughter, II-3, receives the A1B1 chromosome from her father and the A3B3 chromosome from her mother, and is therefore a carrier. In actual fact, II-3 has a very high probability of being a carrier, because sometimes there is a recombination between the X chromosomes in the mother and about 1% (or less) of the time we would be wrong when we say that II-3 is a carrier. Thus, her chance of being a carrier is 99%, using the tests which we have specified. Sometimes the testing is not so good for a particular family because the two X chromosomes in the mother are the same at the A and B loci, and other loci (further from the FMR-1 gene) have to be used as markers.

In Figure 1, we can see that II-3 has two differently marked X chromosomes and she could be offered prenatal diagnosis to determine whether a fetus has a high or low risk of having the defective fragile X gene.

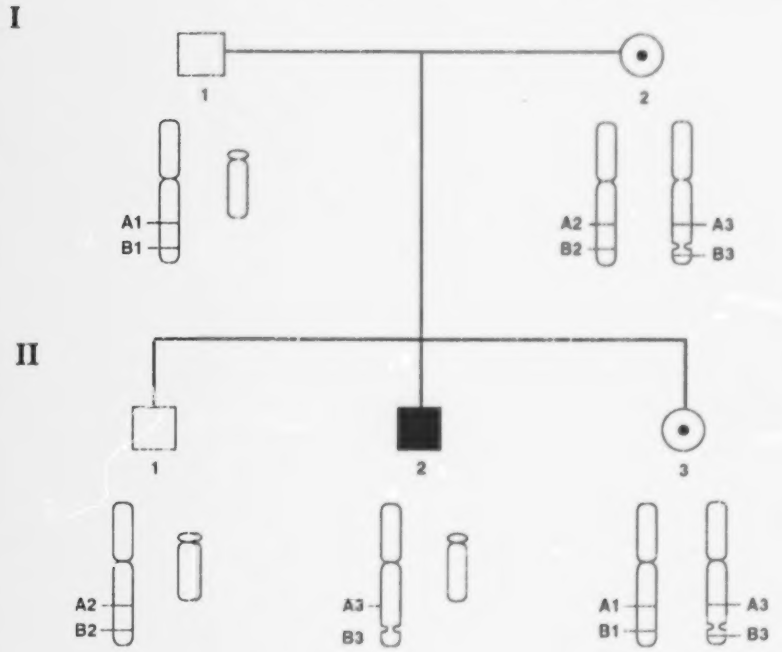

Figure 1: Segregation of DNA markers and FMR-1 gene

DNA studies: The FMR-1 Gene

By May, 1991, three groups (Drs. Stephen Warren, David Nelson and Ben Oostra; Dr. Grant Sutherland; and Drs. Jean Louis Mandel and Isabel Oberle) independently reported the characterization of the fragile X mutation and found that the difference between normal and affected (fragile X) males was the size of the gene! Figure 2A is a diagram of a part of the FMR-1 gene. DNA is made up of 4 building block molecules: A, T, C and G. The sequence of these building blocks defines a gene with a particular function. Genes are usually comprised of thousands of these building blocks. In a small segment of the normal FMR-1 gene, there is a repeat of CGG, such that there are 6-54 copies of this CGG-repeat: CGG CGG CGG CGG etc. In males with the fragile X syndrome, this tiny segment of DNA is increased to 1000 or more CGG repeats. Curiously, the males who "escape" the disease, but transmit the mutation to their daughters, have only 100-200 copies of this repeat. It appears that as the gene gets larger, something happens and it gets turned off: it cannot perform its normal functions and causes what we call the fragile X syndrome.

The current testing for the fragile X syndrome involves examining the DNA for the size of this "variable" region (it is variable in the normal population, too, but only in a small range), as follows (Figure 2B). DNA is isolated from white blood cells and is cut with two special enzymes, each of which recognizes and cuts a specific DNA sequence. We and others use EcoR1 and BssHII, but other investigators use different sets of these restriction enzymes. EcoR1

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cuts DNA every time there is the sequence GAATTC and BssHII cuts every time there is GCGCGC. BssHII can only cut the DNA when it is not modified by methylation (a chemical change to the DNA which occurs at specific sites and is thought to be related to whether the gene is functioning or not functioning). In normal males, then, there are two pieces of DNA (visualized as bands) from the FMR-1 region: 2.4 kb (2400 base pairs or 2400 A,T,C,G's in a row) and 2.7 kb. A normal female has these same two bands of DNA, plus a larger one: 2.4 + 2.7 = 5.1 kb. The larger piece comes from the other X chromosome which is methylated and inactive. The FMR-1 appears to be active on only one X chromosome in females. An affected male has a much larger band for two reasons: 1) there is an increased size of the CGG-variable region, and 2) this increase affects the DNA such that it is modified by methylation and the BssHII enzyme does not cut this section of the DNA. The method for visualizing this is illustrated in Figure 2B. Normal, transmitting males have a larger than normal CGG-repeat, and the band sizes are 2.4 kb and 2.9-3.1 kb. Carrier females also display the abnormal fragments, as illustrated in Figure 2B.

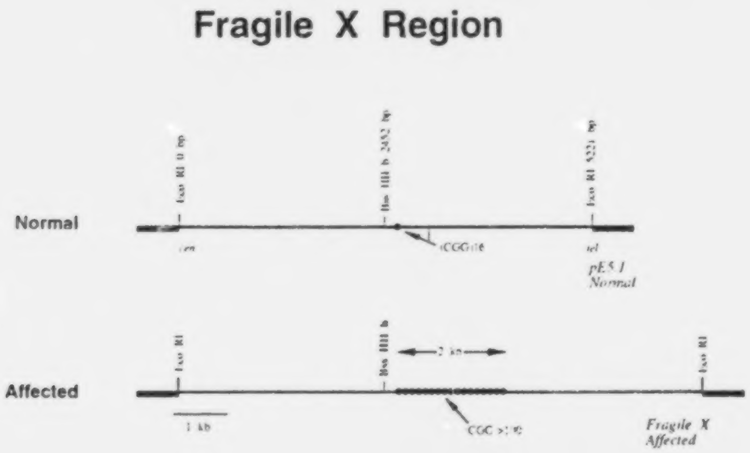

Fragile X Region
Normal
Eco RI 0 bp
Bss HII b 2452 bp
Eco RI 5221 bp
cen
(CGG)16
tel
pE5.1
Normal
Affected
Eco RI
Bss HII b
2 kb
Eco RI
1 kb
CGC >:00
Fragile X
Affected

Figure 2A: Portion of the fragile X gene

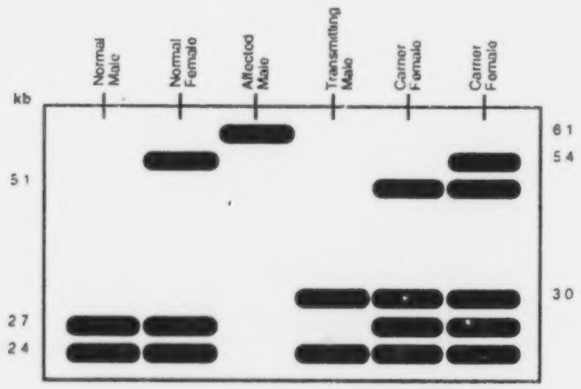

Normal Male
Normal Female
Affected Male
Transmitting Male
Carrier Female
Carrier Female
kb
6 1
5 4
5 1
3 0
2 7
2 4

Figure 2B: Identification of FMR-1 mutations

Another test, PCR (polymerase chain reaction) is also now being used which refines the studies on carrier females. The actual size of the CGG variable region appears to be important in determining the risk that a carrier female has of having an affected son or a son who is a transmitting male (Fu et al, 1991). These studies are still on-going, but it is hoped that the initial findings on some of our families will be extended to confirm or contradict this.

THE SIGNIFICANCE OF THE NEW MOLECULAR TESTING IN THE DIAGNOSIS OF THE FRAGILE-X SYNDROME

This section describes several different questions and scenarios which can now be addressed because of the new tests which allow such precise testing for the fragile X syndrome.

Degree of mental impairment (Figure 3)

Studies are in progress to determine whether there is a relationship between the size of the FMR-1 gene and the degree of mental impairment in males and females.

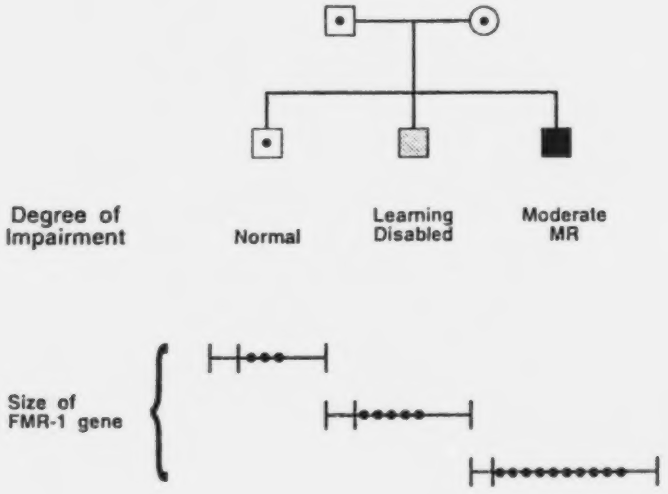


Figure 3: Is there a correlation between the degree of mental impairment and the size of the FMR-1 gene?

Behavioral problems, including autism

There is a wide range of behavioral difficulties in fragile X boys: Is there a relationship to the size of the FMR-1 gene?

There have been varying reports about the association of the fragile X syndrome and autism. Certainly, some autistic individuals do have the fragile X chromosome abnormality. We are now testing a large number of autistic individuals for defects in the FMR-1 gene, because such abnormalities would have implications to other family members.

Depression and Schizophrenia

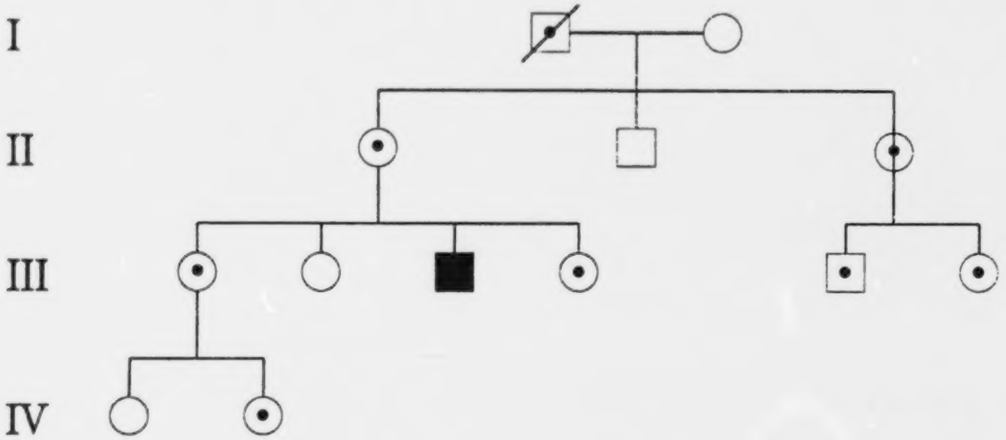
A proportion of carrier females are depressed or rarely schizophrenic. The question is whether this is related to the fragile X syndrome or is an independent problem.

Education

Many males and females with the fragile X syndrome have learning difficulties, resulting in problems with learning specific tasks or skills. Attempts are being made to correlate the size of the gene with difficulties in this area.

Isolated fragile X males (Figure 4)

The new tests now show that ALL mothers (tested to date) of fragile X males are carriers of the fragile X syndrome. This means that other family members are at risk for carrying this mutation and should be tested. The previous methods were unable to show whether another relative was a carrier or not.

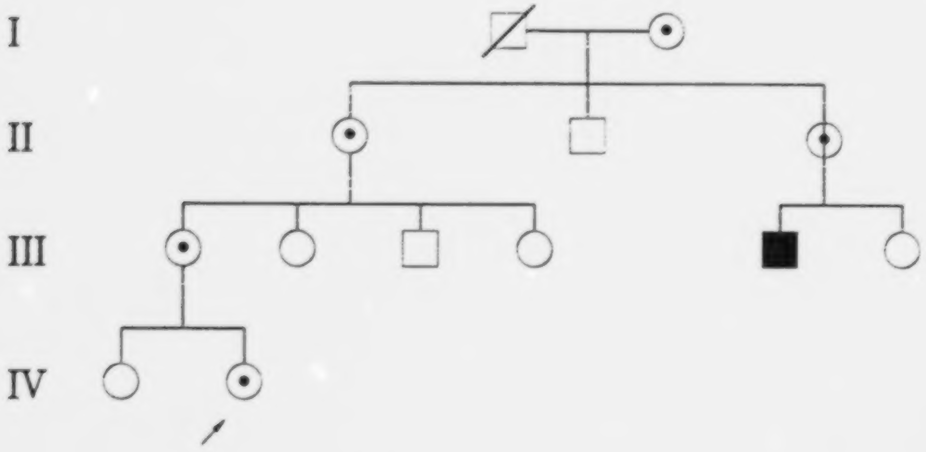


All individuals with a dot in the center were found to be carriers of an FMR-1 gene mutation.

Figure 4: Carrier assessment in families with an isolated fragile X male.

Distant affected relative (Figure 5)

We often have referrals which involve a woman (consultant) who is concerned about a distant male relative who is (or was) developmentally disabled. She is now pregnant and concerned as to whether she has a higher risk for having an affected son or daughter. We can now test the affected individual (or close relatives) and determine whether this individual had the fragile X syndrome. If confirmed, we can then test our consultant and tell her whether she is a carrier or not.

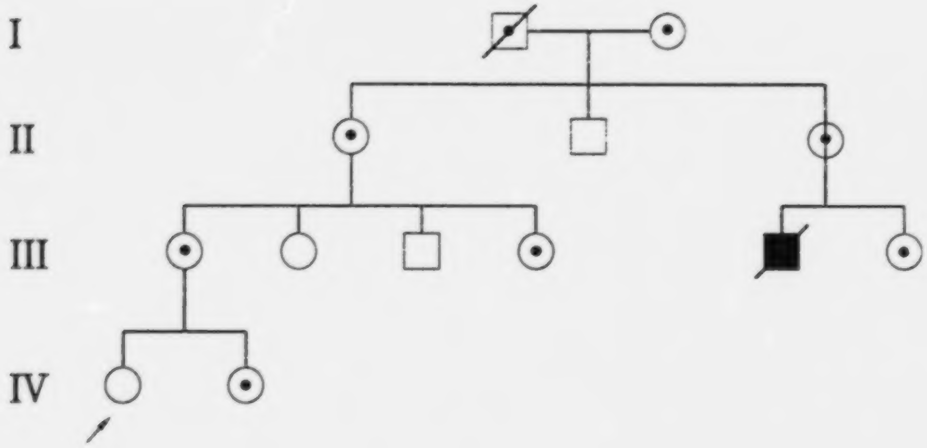


All individuals with a dot in the center were found to be carriers of an FMR-1 gene mutation.

Figure 5: Carrier assessment in families with a distant affected relative

Affected male cannot be tested (Figure 6)

When the affected male or his mother cannot be tested (e.g., deceased, in another country, fear of needles) and there is concern as to whether another family member is a fragile X carrier, the above-described tests can be done. Although we cannot eliminate another cause for the mental impairment, we can confirm whether a person is a carrier of this disorder or not. Since this is the most common form of X-linked mental retardation, it is helpful to be able to determine whether this is a potential diagnosis. In such cases, photographs of the affected individuals may be helpful.



All individuals with a dot in the center were found to be carriers of an FMR-1 gene mutation.

Figure 6: FMR-1 gene testing when the affected male cannot be tested

SUMMARY

I hope that this brief summary of the developments in the study of mental retardation during the past fifteen years has illustrated the importance of both clinical information and laboratory testing, and that without the participation of families and interested researchers in research studies, such advances would not be possible. We have come a long way since the first mention in 1938 of probable inherited forms of developmental disability. Knowledge of the function of the FMR-1 gene will provide us with another dimension in our understanding developmental processes affecting the brain. The next few years will be exciting ones!

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GENETIC COUNSELING ISSUES

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INTRODUCTION

Fragile X syndrome is a genetic condition which causes learning problems ranging from mild learning disabilities to severe mental retardation. Experts now predict that fragile X is the most common single gene disorder known to cause mental impairment. Fragile X syndrome derives its name from the presence of a fragile site or break in the long arm of the X chromosome. The chromosomal finding is frequently associated with a group of physical, intellectual and emotional characteristics. These include intellectual impairment, a long face, prominent or large ears, and poor attention span. Fragile X causes problems in both males and females and occurs among all races and ethnic groups. In addition to the estimated 1 in 1250 males and 1 in 2000 females who have serious problems related to fragile X syndrome approximately 1 in 600 females and 1 in 2500 males are silent carriers of the condition. Silent carriers are individuals who are considered intellectually normal but who are at risk to have children with mental impairment.

FRAGILE X SYNDROME INHERITANCE

Fragile X syndrome is inherited in an X-linked fashion. This means that the gene (a specific segment of DNA) which causes the problems related to fragile X syndrome is carried on the X chromosome. Since a woman has two X chromosomes, she is considered to be a carrier if the fragile X gene is present on one of her X chromosomes. Carriers usually do not have the characteristic features of a disorder because the effects of the fragile X gene are "covered up" by the normal gene on the other X chromosome. However, approximately 1/3 of the carriers of the fragile X gene may have some degree of mental impairment ranging from learning disabilities to mental retardation.

Women who have the gene for fragile X are at an increased risk of having affected children. Each son or daughter of a carrier mother has a 50% chance of inheriting the fragile X gene and therefore has a risk for mental impairment. Since males have one X chromosome, and one Y chromosome, a male who inherits the X chromosome with the fragile X gene will usually be affected. The exception to this are the silent male carriers, (nonpenetrant or transmitting males) who carry the gene for fragile X, but are usually fragile X negative on chromosome analysis. As many as 20% of the males who carry the fragile X gene do not have any or only mild physical, behavioral or intellectual characteristics of fragile X syndrome. For this reason, we call them nonpenetrant. Importantly, males who carry the fragile X gene will pass this gene on to all their daughters but none of their sons.

DIAGNOSTIC TESTING

Chromosome Analysis

Chromosome analysis (sometimes called cytogenetic testing) up until recently has been considered the standard means of identifying individuals with fragile X syndrome. Specialized laboratory techniques are necessary to elicit the fragile X chromosome and even under these conditions, the fragile X chromosome will only be present in a certain percentage of cells analyzed. Individuals who have mental impairment as a result of fragile X syndrome typically demonstrate between 4% and 50% fragile X chromosome expression. Fragile X chromosome analysis will detect approximately 99% of all males affected by the condition and approximately 90% of females who are intellectually impaired.

Chromosome testing for individuals with normal intellectual abilities however, has serious limitations. Approximately 60% of all normally functioning carrier females will not show the fragile X chromosome on cytogenetic tests. Similarly, the vast majority (>99%) of transmitting or nonpenetrant males will test negative on chromosome analysis. Therefore, it is possible to carry the fragile X gene but test negative on chromosome analysis. Because a negative result on chromosome analysis does not rule out the possibility that a person has the fragile X gene, the newly developed DNA testing has become the diagnostic method of choice.

DNA Analysis

The gene which is responsible for fragile X syndrome has been isolated allowing for an increase in our understanding of this uniquely inherited condition. The problems associated with abnormal function of the fragile X gene appear to be related to the number of CGG repeats inserted within the gene. In a non fragile X carrier, the number of CGG repeats is between 6 and 54 with the average number being 29. Repeats which are between 54 and 200 are referred to as premutations and may expand in size when passed from mother to child. An insertion size of 200 or more CGG repeats is called a full mutation. Individuals who carry the premutation tend to be intellectually normal, whereas, males who have the full mutation are usually mentally impaired as are approximately 50% of the females who demonstrate a similar mutation. Importantly, the larger a premutation is in a woman the more likely it is to expand to the full mutation and, therefore, the higher the risk is to have a child with mental impairment. It should also be mentioned that some individuals are classified as mosaics. These individuals have repeat sizes that differ in length. For example, a person may be shown to have a full mutation in some cells but a premutation in others.

Another factor which appears to influence whether an individual is intellectually impaired is methylation. Methylation is a normal process of gene regulation. If a gene is methylated then it is turned off. In the case of individuals with the full mutation, the fragile X gene is methylated when it should not be methylated. Therefore, the gene is abnormally turned off and thus not working as it should. This is thought to lead to a markedly reduced or absent gene product, a chemical which must be critical to normal intellectual development.

It should be noted that there have been a small percentage of families reported who showed the fragile X chromosome but who have not demonstrated the increased CGG repeat (pre or full mutation) nor abnormal methylation. Further work is ongoing to determine whether in fact these families represent another form of fragile X syndrome and, if so, what the molecular mechanism is responsible for the mental impairment in these individuals.

The identification of the FMR-1 gene now allows for more accurate testing. For those families who have had cytogenetic testing or DNA linkage studies in the past, the new direct DNA testing, using techniques called Southern Blot and/or PCR, affords clarification and often more accurate determination of whether or not a person has the fragile X gene. With direct DNA testing analysis, unlike linkage, it is now possible to test any relative with a family history of fragile X syndrome without testing multiple family members. In addition, families who had chromosome studies or linkage studies which were inconclusive, can now have DNA studies performed to determine whether or not their child truly has the fragile X syndrome.

Prenatal studies will also be more accurate using the new DNA tests. Prenatal diagnosis of fragile X syndrome is possible utilizing one or two methods, either chorionic villi sampling (CVS) or amniocentesis. CVS is performed at approximately 9-11 weeks into a pregnancy. A small amount of tissue which would have become part of the placenta is removed. Since this tissue is produced by the developing fetus it contains cells from the baby. Importantly, both chromosome analysis and DNA studies can be performed on these cells. The risk of a miscarriage following a CVS is approximately 1-2%, but may vary according to a center's experience.

In comparison, amniocentesis is routinely performed between 14 and 16 weeks of pregnancy. An amniocentesis is considered a safer procedure than CVS (the risk for miscarriage is approximately 0.5%), however, it has the disadvantage of being performed later in pregnancy. An amniocentesis involves removing a small amount of the amniotic fluid, a fluid which contains fetal cells. As with CVS, chromosome and DNA studies can be performed on these cells.

Direct DNA studies are considered more accurate than cytogenetic studies and DNA linkage analysis for determining carrier status, for performing prenatal studies and when trying to establish the diagnosis of fragile X syndrome within a family. For this reason individuals with a known or suspected family history of fragile X syndrome or mental retardation of unknown etiology are strongly encouraged to seek genetic counseling for the latest information regarding diagnostic testing recommendations.

PSYCHOSOCIAL ISSUES

Support to families needs to be broader than simply providing information relating to testing options and recurrence risks. Validation of individual concerns, acknowledgement of the emotional impact of the diagnosis, i.e., grief, anger, denial, and guilt, and answering questions regarding intervention strategies for an affected child are all appropriately addressed in the genetic counseling session. Families should feel comfortable raising these types of issues and should know that genetic counseling is there for ongoing support and care.

Pregnancy and family planning are common concerns among families with fragile X syndrome. Relatives are strongly urged to meet with the genetic counselor prior to pregnancy to fully discuss reproductive options and available testing. During a pregnancy, whether planned or unplanned, additional issues may surface. For individuals pursuing prenatal diagnosis, close contact with the genetic counselor can provide valuable support. Waiting for test results, dealing with uncertainty and, for some, dealing with sad news can be a trying time. The counselor can be there to help the couple problem solve, help answer new questions that arise and help facilitate the communication process between one partner and the other.

As with other genetic conditions, families with a history of fragile X syndrome are encouraged to seek genetic counseling. Continued follow-up between the families and counselor, though not always possible, is important as it validates the decision making process and outcome. It also allows questions to be readdressed and allows for the overall process to be reviewed as needed. Dealing with the fragile X diagnosis is not easy for most individuals. Genetic counselors are available to provide the added emotional support and understanding many people require. For a genetic counseling center in your area, please contact your family physician or the National Fragile X Foundation at 1-800-688-8765 or (303) 333-6155.

WORKSHOP SUMMARY: MOLECULAR AND CLINICAL CORRELATIONS

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Five presentations in this workshop described a wide variety of experiments aimed toward further elucidation of the fragile X mutation and its effects. Some of the presentations represented additional detail regarding results described in plenary sessions, while for others, this represented the only discussion of the data. Comments and discussion of the results were on the whole quite lively.

Michael Abrams of the Kennedy Institute in Baltimore described efforts to quantify the size and type of mutation in fragile X positive individuals by Southern blot analysis followed by densitometric scanning of bands for length and intensity. These data were then correlated with data collected on the patients regarding their cognition and behavior. A potential correlation between size of insert and IQ was reported, although the data were still very preliminary.

Bert de Vries of Erasmus University in Rotterdam discussed in further detail a case of fragile X syndrome with features of the Prader-Willi phenotype. (Prader-Willi is caused by deletions of chromosome 15 and causes childhood obesity thought to be due to obsessive over-eating). This patient exhibits a deletion of the region of the FMR-1 gene in a portion of his blood lymphocytes, while the remainder of the cells show the typical large insert at the CGG repeat region. The deletion involves some of the repeats as well as a significant (~250 bp) stretch of adjacent DNA. Another intriguing finding was the higher proportion of younger fragile X males with "mosaic" mutations relative to older males. These data were interpreted to signify continuing instability of the repeat sequences during life, resulting in the alterations of the proportion of cells with different sizes of repeats over time. One interpretation of these data is that many fragile X males begin life with smaller, premutation alleles which gradually convert to full mutations with age. This would imply that the transition from premutation to full mutation occurs post-conception, however there is no direct evidence of this.

Karen Snow of the Mayo Clinic presented results of her surveys of fragile X and normal families with regard to methods of determining allele sizes. She discussed difficulty with the Brown method of detecting full mutations after PCR amplification and with the Fu method with respect to mosaic individuals. Four families without the standard repeat expansion were presented; these were problematic diagnostically, but may represent other mutations.

Premutation alleles in fragile X families in this study ranged from 57 to 150 repeats. Risks of full mutations in mothers with premutations were determined; these varied slightly from the data of Fu et al. Similarities included a 90 repeat threshold above which all alleles expanded to full mutations, nearly identical risks in the 81-90 repeat range (86 vs. 82%), and the finding of no expansions of alleles at or below 60 repeats. However, in the 61-70 repeat range, double the frequency of Fu (33 vs. 17%) was reported, while in the 71-80 repeat range, only 47% of alleles expanded (vs. 71% in Fu).

In normal families, a range of repeat sizes between 10 and 51 repeats was found, with familial stability observed in four high-end alleles (51, 50, 47 and 45 repeats), one of which (51 repeats) could be tracked for four generations. A population of anonymous individuals consisting of 396 alleles revealed three alleles in the "grey zone" of 61, 57 and 52 repeats. No data on familial stability of these repeats could be gathered due to the anonymity of the subjects.

Francois Rousseau of Universite Laval in Quebec presented results of a large collaborative study of fragile X families using the StB12.3 probe and Eco RI + Eag I digestion carried out worldwide. A total of 235 pedigrees involving 2216 individuals were studied in 13 laboratories. Correlative studies of the Southern blot results with a variety of physical and mental data were carried out. Intriguing among these were the identification of several cases of mental retardation in male and female carriers of the premutation. Among individuals demonstrating a full mutation, four males (of 182) and 52 females (of 133) showed no mental impairment.

A poor correlation between size of increased repeat band and percent of cells demonstrating cytogenetic expression was reported, along with no correlation between insert size and degree of macroorchidism. The facial features of fragile X syndrome appeared to be less pronounced among mosaic individuals. Important for prenatal studies was the finding that 10 of 12 chorionic villus samples from affected fetuses were found to be methylated at the Eag I site, while all amniotic fluid samples showed methylation.

Charles Laird of the University of Washington, Seattle, described his theory of fragile X syndrome's genesis, and data testing one of its predictions, namely that the "imprint" in a fragile X male would be "erased" in his daughters. Two families in which an affected male had fathered daughters were studied, and in each the daughter exhibited alleles of the premutation type (i.e., deltas of 200 or 250 bp.). One of these daughters went on to have two affected sons, both of whom demonstrate full, methylated mutations. Two explanations for the observation of reduction in allele size were proposed: 1) the full mutation cannot be propagated in the germ line, thus the only X-bearing sperm produced are of the premutation variety or 2) that the full mutation actually is never found in the germ line, and that the expansions found in somatic tissue are not representative of the alleles present in germ-line tissues. Experiments to test these alternatives were discussed.

GUIDELINES FOR THE DIAGNOSIS OF FRAGILE X SYNDROME

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INTRODUCTION

Fragile X syndrome is the most common inherited form of mental impairment, affecting approximately 1 in 1250 males (Brown, 1990). The disease is associated with the expression of a folate sensitive site at Xq27.3 although the level of expression can vary from a few percent to as high as 70%. Recently, cloned DNA sequences which span the fragile site at Xq27.3 have been isolated (Oberle et al., 1991; Yu et al., 1991). At this site the FMR-1 gene has been identified (Verkerk et al., 1991) containing an unstable region of DNA which segregates with the fragile X phenotype (Oberle et al., 1991; Yu et al., 1991; Verkerk, et al., 1991; Kremer et al., 1991; Rousseau et al., 1991). The unstable DNA was found to involve the repeat sequence $(CGG)_n$. A correlation between the length of the unstable sequence and the phenotype is observed, thus permitting reliable and accurate diagnosis of fragile X carriers and patients by direct molecular analysis (Oberle et al., 1991, Yu et al., 1991; Verkerk, et al., 1991; Kremer et al., 1991; Rousseau et al., 1991).

Until quite recently, the diagnosis of fragile X syndrome was based on the cytogenetic detection of a fragile site at Xq27.3. Numerous factors including specific tissue culture requirements, the type of cell population being studied, the degree of involvement and sex of an individual patient, contributed to the reliable cytogenetic detection of the fragile site and the diagnosis of the syndrome. An Ad Hoc Committee was convened by Jacky at the Fourth International Workshop on the fragile X syndrome to recommend minimum cytogenetics standards for the preparation and analysis of the fragile X chromosome (Jacky et al., 1991). The guidelines described criteria for tissue culture methods for induction of the fragile site in vitro and for the analysis and interpretation of such chromosome preparations.

Direct DNA analysis of the mutations responsible for the clinical expression and transmission of the fragile X syndrome has begun to replace the cytogenetic analysis. This transition reflects both the generally cheaper cost of establishing the diagnosis in individuals and through families with molecular methods, and on the better reliability of DNA testing in establishing carrier status for the premutation in female and some male individuals. The fragile X mutation is considered to be due to elongation of a small DNA sequence containing a repeat of the trinucleotide CGG located in a 5' exon of the FMR-1 gene (Oberle et al., 1991; Yu et al., 1991; Verkerk, et al., 1991; Kremer et al., 1991; Rousseau et al., 1991; Fu et al., 1991). The increase of the number of repeats can be determined either by Southern blot analysis of genomic DNA or by PCR analysis of the CGG repeat. During the Third International Fragile X Conference in Snowmass, Colorado (1992), a protocol was developed and adopted to better define the evolving relationships between cytogenetic and molecular testing for fragile X syndrome.

CYTOGENETIC DIAGNOSIS

The Snowmass meeting generally acknowledged that we have entered a period of validation between conventional cytogenetic fragile X testing and molecular evaluation, and a number of reference laboratories have been encouraged to continue these studies in parallel for validation purposes, especially for prenatal diagnosis. It should be clearly acknowledged, however, that in the absence of the specific fragile X cytogenetic testing, a routine constitutional chromosome analysis will remain an integral part of the diagnostic workup of any patient, male or female, demonstrating mental retardation or significant developmental delay. These studies are appropriate on an individual independent of a family history of intellectual handicap, or obvious fragile X physical stigmata (Jacky and Jenkins, 1992). This recommendation reflects the occurrence of a large number of other constitutional type chromosome abnormalities that have been detected when patients were referred for fragile X analysis.

Fragile X chromosome studies under some institutional protocols may remain the primary diagnostic tool in defining the initial propositus in newly diagnosed families, to be followed then by a full molecular evaluation of the sibship. However, in general DNA analysis will become the diagnostic tool to identify the proband in newly diagnosed families. Fragile X chromosome studies will in any case remain important in terms of understanding the significance of frequencies of fragile site expression in individuals and fragile site chromosome structure events as they relate to changes at the molecular level.

METHOD OF DNA DIAGNOSIS

A CpG island described in Xq27.3 has been shown to be abnormally methylated in affected individuals (Vincent et al., 1991; Bell et al., 1991). Several probes can be used to detect this abnormal methylation (Figure 1) (Rousseau et al., 1991). At the same time an increase in size of a fragment containing the CpG island and a CGG repeat can be detected. For example, in normal individuals a 5.2 kb EcoRI fragment is detected (Table 1). In affected individuals an increase of this fragment is found with a length generally above 5.7 kb. In unaffected transmitting male carriers and a portion of female carriers, a premutation allele between 5.3 - 5.7

kb is detected. Table 3 shows the different fragment sizes detected after digestion with different restriction enzymes.

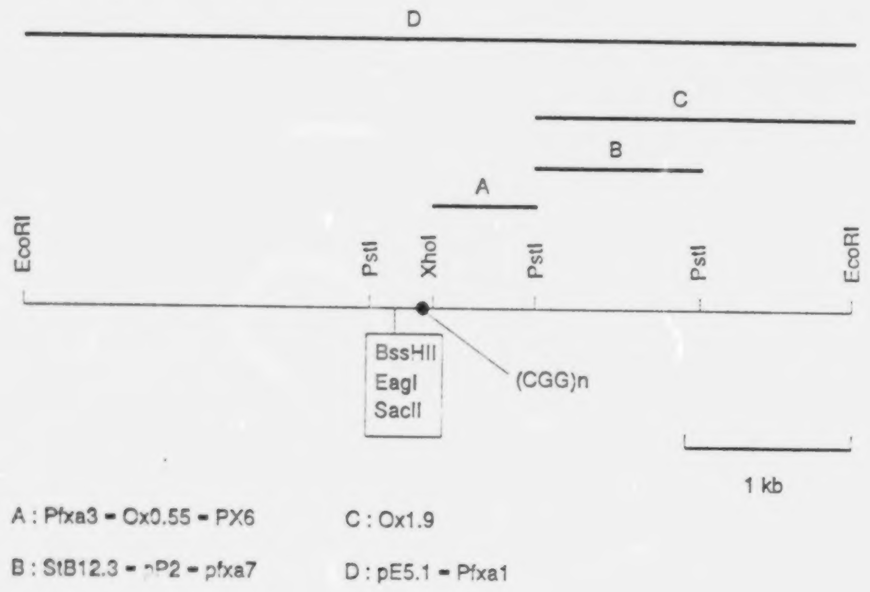
D
C
B
A
EcoRI
PstI
XhoI
PstI
PstI
EcoRI
BssHII
EagI
SacII
(CGG)n
1 kb
A : Pfxa3 - Ox0.55 - PX6
C : Ox1.9
B : StB12.3 - pP2 - pfxa7
D : pE5.1 - Pfxa1

Figure 1. Probes used in the diagnosis of fragile X syndrome. The restriction map of the 5.2 kb fragment containing the fragile site, the location of the CpG island (boxed), and the CGG repeat is given. The location and the names of the restriction fragments used to characterize the fragile X genotype are indicated.

Table 1
Restriction Fragment Size (kb)

	EcoRI[a,d]	**EcoRI + EagI[a,d]**		**BglII[a]**	**PstI[b]**	**PCR[c]**
		♂	♀			
normal	5.2	2.8	2.8+5.2	12	1.0	6-51
premutation	5.3-5.7	2.9-3.3	2.9-3.3	NR	1.1-1.6	48-200
full mutation	> 5.7	> 5.7	> 5.7	> 12.5	NR	> 200

a. 0.7% agarose gel using probes B or C (Figure 1). b. 1.0% agarose gel using probe A (Figure 2). c. PAGE. d. partial EcoRI digest will generate a band 1.2 kb larger than the expected size(s). NR: not recommended.

We propose for the detection of methylation of the CpG island the use of a double digest of EcoRI and the methylation sensitive enzyme EagI. Using a probe distal to the CpG island (Figure 1, probes B or C) the normal fragments are 2.8 kb in males and females and 5.2 kb on the female inactive X chromosome. The sizes of the EcoRI+EagI fragments for the premutation and full mutation alleles are given in Table 1.

Variation in the mutation of the FMR-1 gene can be detected after digestion of the DNA with different restriction enzymes. The choice of enzyme to use is dependent on the size of the insert. The detection of the small insert found in premutation alleles can be carried out with great accuracy using the enzyme PstI. Full mutations are on the other hand often heterogeneous, resulting in a smear of bands. Mutations in fetal tissue are especially heterogeneous and we recommend performing a BglII digest (Rousseau et al., 1991). The smear of bands will be compressed at the top of the gel with optimal detection of the full mutation.

As an alternative in molecular diagnosis, PCR analysis of the CGG repeat can be carried out. Two slightly different methods have been described by Fu et al. (1991) and by Pergolizzi et al. (1992). The method of Fu et al. (1991) is applicable for the amplification of small alleles, but the amplification of larger (full size) alleles is so poor that they are undetectable. The PCR method described by Pergolizzi et al. (1992) employs a different amplification protocol and uses a hybridization step with a CGG repeat probe to detect the amplification products. Initial results (discussed at the conference, W. T. Brown) suggest that, using either PCR protocol, the resolution by polyacrylamide gel electrophoresis of the shorter PCR products in the normal and premutation range is finer and allows for better size determination, whereas the use of agarose gel electrophoresis may allow for better visualization of full mutations. The mosaicism observed in some individuals appears to be related primarily to methylation status of the CpG island which is not detected by PCR. When a large premutation or a (small) full mutation is detected in order to determine if mosaicism is present, complementary analysis by direct Southern blotting employing digestion with a methylation sensitive enzyme such as EagI is recommended. Further, when affected females with a normal allele size and a full mutation are suspected, selective amplification of the smaller allele may result in a single normal allele size and interpretation may be difficult. Such homozygous allele PCR results also should be checked by direct analysis.

DIAGNOSTIC PROTOCOLS

We propose different protocols for prenatal and postnatal diagnosis (Tables 2 and 3). Postnatally, a thorough diagnostic evaluation of a mentally retarded individual without a family history of FRAXA requires the molecular demonstration of the full mutation, or the presence of a chromosomal marker abnormality responsible for the intellectual handicap. Fragile X cytogenetic testing under this circumstance may no longer be necessary. Individuals with a family history of mental retardation with or without FRAXA physical stigmata follow a similar diagnostic format but may under this circumstance include FRAXA cytogenetic studies for validation purposes. Frequently in FRAXA pedigrees the diagnostic testing has to be extended to a large number of individuals, and must discriminate between normal alleles, premutation alleles and full mutation alleles (Table 1). There is not an exact cutoff between large premutations and small full mutations (in the 200 repeat region). The presence of abnormal methylation allows one to identify the full mutations when the size is borderline. Special attention has to be given to the diagnosis of the premutation allele that has an amplification of the CGG repeat between 48 and 200 CGG repeats. As is shown in Table 3, a region of overlap is found between the smallest premutation alleles and the largest alleles found in normal individuals, approximately 50-60 repeats. Molecular evaluation of extended fragile X family members generally allows discrimination between normal and premutation alleles. In population screening it may be impossible to distinguish between normal and premutation alleles in this range.

Table 2
Postnatal Diagnosis

A. Mental Retardation	a. unknown etiology	1.	constitutional chromosome analysis	
		2.	DNA	EcoRI digest PCR P[a]
	b. family history (MR) without FRAX physical stigmata	1.	DNA	EcoRI digest
		2.	constitutional chromosome analysis	
		(3.	optimal FRAX chromosome analysis)	
B. Carrier testing (family history of FRAXA)			DNA	a. EcoRI+Eagi digest b. PCR F[b] or PstI digest or PCR P[a]

a. Pergolizzi et al. (1992); b. Fu et al. (1991)

Table 3
Prenatal Diagnosis

A. DNA
  a. EcoRI + EagI digest or PCR[a]
  b. BglII digest
  c. PCR P[b] or F[b] or PstI digest[c]
  As control: CA microsatellites (close to FRAXA)
B. Constitutional chromosome analysis[d]

a. Pergolizzi et al. (1992); b. Fu et al. (1991); c. In case of diagnosis normal transmitting male in chorion villi confirmation on amniotic cells or fetal blood is advised; d. In a number of centers a cytogenetic test of fragile X will be carried out to validate prenatal DNA testing.

A modification of this protocol is proposed for the prenatal diagnosis of fragile X syndrome (Table 2). Full mutations can be detected after analysis of an EcoRI+EagI digest, but it has to be noted that the methylation status in chorionic villi can be different from actual fetal tissue and the full mutation may be unmethylated. The mutations in fetal tissue are very heterogeneous. We recommend performing also a BglII digest. Premutation alleles can be detected by PCR or PstI digest. PCR analysis of flanking microsatellite CA repeats (Richards et al., 1991; Riggins et al., 1992) can be carried out as a control and enables rapid detection of the normal genotype in more than half of at-risk pregnancies.

To date, the number of prenatal diagnoses analyzing the number of CGG repeats is relatively small (Hirst et al., 1991; Dobkin et al., 1991; Sutherland et al., 1991; Devys et al., 1992). At the Third International Fragile X Conference in Snowmass, Colorado, it was proposed that a number of laboratories continue to carry out cytogenetic testing of the fragile site as a validation of DNA testing in prenatal diagnosis (see reports by Jenkins, Howard-Peebles and Shapiro in these proceedings). Data for the validation will be collected and presented during the next X-linked Mental Retardation meeting in 1993.

Molecular prenatal diagnosis is further confounded by other not well-understood factors. For instance, the mechanism of the extension from premutation to full mutation is still unknown. It is also not known when this expansion takes place. Therefore, one should be very careful in predicting whether a premutation found in chorionic villi accurately reflects the mutation pattern in fetal tissue. Until more data is available we recommend confirming the diagnosis of a premutation using CVS by subsequent analysis.

REPORTING OF DNA DIAGNOSTIC RESULTS

The size of the CGG repeat and the presence of abnormal methylation appears to determine the phenotype. A normal phenotype is predicted for individuals with a premutation. Males with a full mutation (and abnormal methylation) always show the fragile X phenotype with mental retardation. The same phenotype is usually observed for males with a mosaic DNA pattern. Insufficient data is available to predict the phenotype of females with a full mutation. From the available data, the risk of showing mental impairment for females with a full mutation can be predicted to be between 50-70% (Table 4).

Table 4
Reporting

DNA test	**phenotype**	
normal	normal	
premutation	carrier with normal phenotype	
full mutation[a]	male:	high probability of mental impairment
	female:	50-75% probability of mental impairment 25-50% carrier with normal phenotype

a. including mosaics

CONCLUDING REMARKS

Direct DNA analysis of the fragile X mutation has become available with the isolation of DNA probes that detect the unstable DNA sequence containing the CGG repeat. We have presented strategies that can be applied for postnatal and prenatal diagnosis of fragile X syndrome. In the protocols we have combined the different probes and enzymes combinations that were presented in a number of papers (Oberle et al., 1991; Yu et al., 1991; Verkerk, et al., 1991; Kremer et al., 1991; Rousseau et al., 1991; Fu et al., 1991; Pergolizzi et al., 1992; Hirst et al., 1991; Dobkin et al., 1991; Sutherland et al., 1991; Devys et al., 1992; Mulley et al., 1992). Using the guidelines a reliable postnatal and prenatal diagnosis of fragile X syndrome can be carried out. Special precaution has to be taken in case of a diagnosis in CVS of a premutation in a male fetus. Until more data is available, confirmation of the diagnosis in amniotic fluid cell is recommended.

Direct DNA analysis of fragile X mutation by molecular analysis has already proven itself superior for diagnosis of the fragile X syndrome. Direct DNA analysis brings to an end the era of uncertainty of the fragile X diagnosis for patients and carriers.

ACKNOWLEDGEMENTS

The authors are indebted to the National Fragile X Foundation for organizing the Third International Fragile X Conference in Snowmass, Colorado that served as a platform for discussion with our colleagues which resulted in these guidelines.

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APPLICATIONS OF MOLECULAR TECHNOLOGY FOR CLINICAL USE IN THE FRAGILE X SYNDROME

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The recent identification and characterization of the molecular region associated with the fragile X mutation has propelled us into a new era in our diagnostic testing approaches and ability to screen for the fragile X syndrome. There are now new technologies available to assist families with carrier screening and prenatal diagnosis. Chief among these are the ability to analyze the fragile X gene, FMR-1, using direct Southern blotting and the ability to employ polymerase chain reaction (PCR) technology. We would like to summarize some developments in technology and their clinical applications we have recently studied. We will indicate where these advances are likely to lead. As this article is intended for a general audience, original references and technical details are minimized. We will assume the reader is familiar with standard terms used in other articles summarizing conference presentations dealing with molecular approaches.

The clinical question we have most frequently been asked over the last several years is whether the sisters of a fragile X affected male are carriers. Until the recent testing methods were developed, providing genetic counseling to sisters was often particularly difficult. If a sister of a fragile X positive male sought counseling, we would say that her prior risk of being a carrier was 50:50. If she had fragile X cytogenetic studies which were negative, and if she was unaffected intellectually within the family context, we would counsel her that, based on the epidemiological studies of Sherman et al. (1984, 1985), her risk would be reduced to about 30%. This was still a high risk. This risk could be reduced even further provided her family could be tested with DNA linkage analysis and informative markers found. In the period of time from 1984 to 1991 as closer and closer flanking DNA polymorphic markers were isolated, the probability of flanking markers being informative and of being able to give reliable risk figures became higher. In addition to sisters, it was possible to also look at the presumably normal brothers of affected males to determine if they were non-penetrant male carriers. We have carried out a linkage study of 92 unaffected brothers (Brown et al., 1991). Using the available probes, we found 51 were doubly informative with flanking markers on both sizes of the fragile X locus. Of these we found that 10 (19.6%) were non-penetrant males, thus confirming the predicted frequency based on Sherman's epidemiological studies. However, there always seemed to be a few sisters and brothers for whom we were unable to estimate reliable risk figures due to recombination of the closest flanking markers.

This situation rapidly changed in 1991 as the nature of the fragile X mutation became evident (Verkerk et al., 1991) and as fragile X direct probes became available. Mandel and Rousseau kindly made their probe, StB12.3 (Rousseau et al., 1991), available to us and we have much experience with its use. We were first to employ this probe for direct prenatal diagnosis (Dobkin et al., 1991). However, even for direct analysis, a significant amount of DNA (6-10 micrograms) is required to perform the test. Typically, from a CVS sample of 20 milligrams' weight, about 20 micrograms of DNA is isolated. This is usually an insufficient amount to use for direct DNA analysis as well as to have material to conduct cytogenetic studies. Thus, we generally have to culture either amniotic fluid or chorionic villus samples for two to three weeks prior to obtaining a sufficient number of cells for DNA isolation and analysis to ensure a reliable result. Further, the DNA analysis typically takes a week to ten days. In order to speed up the time, we have been working to develop improved testing using PCR.

PCR analysis is a significant new technology being used for analysis of a variety of human genetic mutations. It has a number of advantages. It is fast, efficient, inexpensive and can be performed with very small amounts of DNA. In brief, the method of PCR is as follows. A double stranded DNA molecule, the template, is isolated from the person being studied and is denatured by heating to a temperature where the two complementary strands separate. Small primers, short complimentary DNA strands approximately 20 bases long are used which exactly match the DNA above and below the CGG repeat region. They hybridize to their complimentary sequence on the single stranded template DNA. An enzyme, DNA polymerase, uses a primer attached to its corresponding template to synthesize a new double stranded DNA. Starting with one copy of DNA a duplicate is created in the test tube. This is one cycle which typically lasts about five minutes. It is carried out in a machine, an automatic thermocycler, designed to heat and cool in a cyclic fashion. Each cycle doubles the amount of DNA present. After 30 cycles, the amount of DNA present has increased to 2^{30} copies or about 1,000,000,000 times more DNA than the starting amount.

Initial attempts to amplify the CGG repeat region in the fragile X locus showed this was a difficult region to amplify (Yu et al., 1991). Nelson's laboratory (Fu et al., 1991) published a method which successfully amplified normal sized alleles and most premutation alleles but failed to amplify the full mutation. We developed a method which successfully amplified the full mutation (Pergolizzi et al., 1992). The differences in our method and that of Nelson's were primarily the use of 100% 7-deazaguanine instead of 75% and the use of a CGG repeat oligonucleotide probe for detection of the amplified product instead of internal labeling.

We have continued research aimed at improving and optimizing the methods for PCR analysis of the fragile X mutation. We have modified the protocol we are currently using from what was published in a number of ways. These include 1) the use of new optimized primers, 2) the use of a new temperature and time-cycle profile which improves the amplification product, 3) optimization of various concentrations of amplification reaction ingredients, 4) the use of denaturing polyacrylamide gels which give better separation of normal alleles, 5) the use of electroblotting to speed up transfers, and 6) the use of a rapid non-radioactive detection method using a Lumi-Phos 480 labeled oligo-probe (supplied by Lifecodes, Inc., Stamford, Conn.). These modifications have reduced the time necessary to obtain results. Starting with isolated DNA, the amplification typically takes approximately three hours, the electrophoresis approximately one hour, the transfer approximately one hour, the hybridization procedure approximately one hour, and the file exposure and development approximately one hour. Allowing time for lunch, we can start the amplification in the morning and have the results within eight to ten hours, although we generally do the amplification on one day or over night and the analysis the next day. In reality, the procedure for the isolation of DNA takes longer than the analysis. This is because we have found that the standard method involving phenol-chloroform extraction gives us the most satisfactory DNA for amplification of prenatal samples.

Our current protocol involves an initial screen by PCR of any samples we received. We calculate the allele sizes relative to a set of standard molecular weight markers or by a comparison to a mixture of three female DNA samples producing six different size alleles that we have determined previously. If it is a male and we see a normal allele size, we stop. If it is a female and we see two normal alleles, we stop. Otherwise, if the sample shows an affected pattern, or in the case of a female appears homozygous, we proceed to screen by direct Southern blotting using probe StB12.3 to be sure that we are not missing a large full mutation which failed to visualize. For the present, we are also conducting cytogenetic studies and direct studies for validation purposes in all prenatal diagnoses.

To date, over 250 specimens, including 50 pregnant women referred to detect fragile X status, have been tested using this investigational protocol. We find good agreement with previously published normal allele distributions as well as with direct probe and cytogenetic analysis. Initial PCR analysis of CVS samples within three days has been accomplished in some cases. With further experience, we believe this PCR protocol can be applied to widespread population screening and to assist with prenatal diagnosis.

To illustrate the results we obtain with this procedure, Figure 1 shows the detection of a normal male fetus by CVS. The CVS procedure was done on a Monday and we received the specimen on a Tuesday. After DNA extraction, on Thursday we performed PCR amplification of the CVS along with a sample of DNA from the parents. That Friday afternoon we had the results, three days after receiving the specimens. The mother had a normal allele of 30 repeats and a premutation allele of 90 repeats. The husband had 29 repeats. The fetus, which was determined to be male by the use of X and Y specific PCR analysis, had a single allele of 30 repeats. These results were confirmed both by cytogenetic and direct DNA analysis four weeks later. Figure 2 shows an amniotic fluid sample set to us which was obtained at 19 weeks of pregnancy from a mother who had a retarded brother who was unavailable to test. We analyzed both the mother and the fetus at the same time and found the female fetus had two normal alleles as did the mother. The figure shows the use of a mixture of three heterozygous females as a marker of allele sizes.

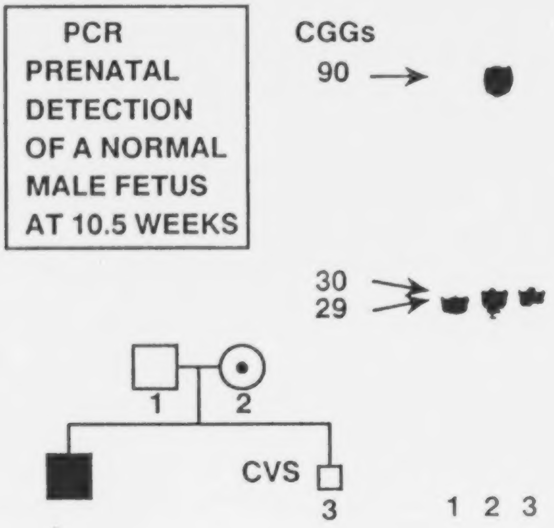
PCR
PRENATAL
DETECTION
OF A NORMAL
MALE FETUS
AT 10.5 WEEKS
CGGs
90
30
29
1
2
CVS
3
1 2 3

Figure 1

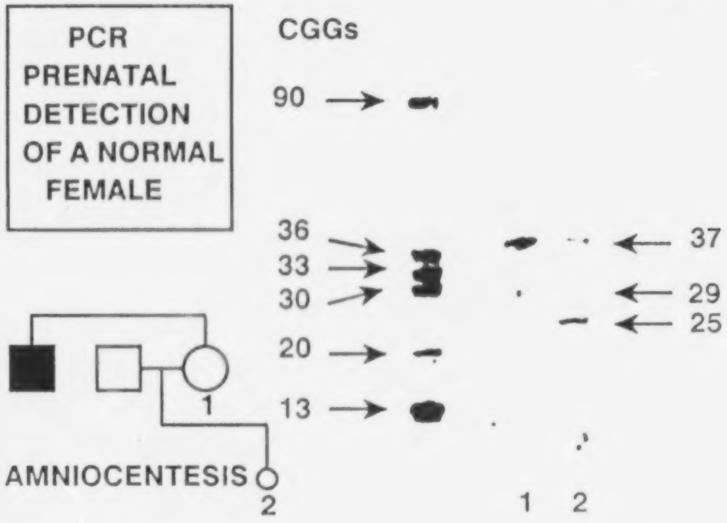
PCR
PRENATAL
DETECTION
OF A NORMAL
FEMALE
CGGs
90
36
33
30
20
13
37
29
25
1
AMNIOCENTESIS
2
1 2

Figure 2

With further experience, we believe the PCR analysis can be applied to routine screens. Currently, we estimate the costs of materials, labor and handling per sample to be approximately $50, whereas the costs of direct probe analysis generally runs about $200 per specimen. In the near future, it would appear reasonable to recommend all pregnant women be screened. Also, all developmentally disabled children who are undiagnosed could be screened. In principle, a dried blood spot could be analyzed from all newborns as well. Technically this seems quite feasible. It has been shown that a dried drop of blood yields about 1,500 nanograms of DNA. We have successfully amplified less than one ng of DNA using our current protocol. However, screening raises difficult issues of confidentiality, access to information, and insurability as Dr. Sherman has addressed in her presentation.

There are several questions that need to be addressed regarding screening of the general population. Firstly, how common are carriers? Secondly, what does the identification of a carrier mean? As has been shown, there appears to be differing risks of transition to the full mutation based on the size of the premutation (Fu et al., 1991). Female carriers of a premutation of >90 repeats have a very high rate of progression to the full mutation. Carriers of premutations in the range of 60-70 have perhaps a 20% risk of progression. But what about the range of 50-60? The frequency of individuals with repeats in this range and their degree of instability will need to be worked out by population screening programs and by detailed family studies. Correlations between the molecular mutation and clinical phenotype will also need to be addressed. At present, the phenomenon of mosaicism which occurs in affected males and females at a rate of 15% (Rousseau et al., 1991) is of great interest. We are in the process of reanalyzing a family (F111) with collaborators from Duke University that we previously studied in which there were three affected brothers and three normal brothers. Two of the normal and two of the affected brothers were found to be mosaics and it appears there exists a gradient of disability related to the degree of mosaicism. However, we doubt the molecular situation will give us as much predictive power as we would like. For example, in recent follow-up studies of prenatally detected positive females including retrospective molecular analysis, we have shown that the risk of mental impairment is about 75% (Brown et al., 1992) in females with the full mutation.

We would like to turn now to a discussion of some ideas regarding mechanisms and hypotheses. A recent collaborative study of Richards et al. (1992) to which we contributed suggests the existence of a fragile X founder effect indicating there may be ancestrally old X chromosomes on which the full mutations arise. It appears the fragile X mutation is not a single step change, but represents a multistep change. It appears that the transition rate from a normal sized allele of less than 50 repeats to one of approximately 50-59 repeats is on the order of 1/1000 gametes, that the transition rate from 50-59 repeats to the range of 60-69 is on the order of 1/10, while the transition rate of 60-69 to a greater size is high, and the transition from premutation alleles of >70 is very high. The mechanism by which such transitions arise may be by a mechanism of slippage similar to that described by Levinson and Gutman (1987). Variations in the underlying CGG repeat region sequence (it appears from the sequences currently available that perhaps 1 in 10 Cs are replaced by As) could relate to the founder chromosome effect by significantly altering the rate of transition by a mechanism such as slippage.

The effect of the full mutation appears to be the lack of synthesis of a gene product of the FMR-1 locus. At present, the function of this gene product is unknown, but since it is highly evolutionarily conserved it must play an important basic role. Based on recent work suggesting an abnormality of adenyl cyclase levels in fragile X (Berry-Kravis and Huttenlocher, 1992), we speculate it is a regulatory protein which in some way determines the level of expression of a particular adenyl cyclase. Four different adenyl cyclase genes have been cloned to date. Perhaps one has a critical role in regulating the structure of dendritic spines. Lacking such activity may lead to immature spine morphology such as we have described in fragile X brain material (Hinton et al., 1992). This abnormality could have profound effects on learning, memory and behavior. Our goal in the future should be to apply the available technology to come to an understanding of the underlying molecular nature of the defect in the fragile X brain and to determine if and how it can be treated.

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DIRECT DNA TESTING AT THE FRAGILE X LOCUS: EXPERIENCE TO DATE, THE IDENTIFICATION OF OTHER MUTATIONS AND SITES OF CHROMOSOMAL FRAGILITY

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INTRODUCTION

The fragile X syndrome is the most common single recognized form of inherited mental retardation. We now have a basic understanding of the mutation responsible for the syndrome, and can apply this knowledge in its diagnosis and hence, its counseling. A gene identified at the fragile X site and mutations within it provide an explanation for the unusual inheritance patterns of the fragile X syndrome and give insights into the puzzling genetics of other common genetic disorders. In brief, the fragile X mutation is due to the presence of an unstable CGG repeat region of DNA within a gene called FMR-1 (for Fragile X Mental Retardation). The region of unstable DNA gradually increases in length and in degree of instability in successive generations until a critical point is reached where the gene can no longer function correctly. The consequence of such genetic instability is the clinical manifestation of the fragile X syndrome. In this report, we summarize our work to date with diagnosis and the study of exceptional cases.

DIAGNOSTIC STRATEGY

For diagnostic purposes, molecular changes at the FMR-1 gene can be used as a direct test for the prediction of clinical phenotype (Nakahori et al., 1991; Hirst et al., 1991a; Hirst et al., 1991b; Knight et al., 1992; Snow et al., 1992). Unlike many other human genetic diseases, these tests are unusual in the fact that the interpretation of many of the results is complicated by the very nature of the mutation itself.

The length of the unstable region of DNA can now be determined in an individual using DNA probes in standard laboratory tests. These are very specific and sensitive methods of making the diagnosis of the fragile X syndrome. They are, more importantly, very sensitive for identifying carrier males and females, not previously possible cytogenetically. The various tests available make use of different restriction digests and DNA probes. The strategy for Southern blot diagnosis, based upon the experience in the Oxford group, is outlined below.

Restriction enzyme	Probe	Detects
EcoRI or HindIII	Ox1.9	All affected males > 90% small insertions
PstI	Ox0.55	Size all premutations Normal allelic variation
BglII	Ox1.9	Heterogeneous alleles
EcoRI+BssHII or EagI	Ox1.9	Methylation status of FMR-1 HTF island

The use of the single EcoRI or HindIII digestion in affected male individuals will identify the full mutation in >99% of cases. The exceptions to this rule have been either mosaic individuals or those who have a single fragment in the premutation range that is fully methylated (Knight et al., 1992). All small increases in fragment size and individuals having apparently normal sized alleles are further investigated with the PstI assay. This serves to size small premutations accurately. The BglII assay is used to resolve the heterogeneous full mutation when not resolved in EcoRI or HindIII assays (Nakahori et al., 1991; Hirst et al., 1991a). It serves to sufficiently compress the signal so as to give a detectable signal on a Souther blot.

PCR BASED TESTS

An essential addition to this array of tests has been the use of tightly linked (CA) polymorphic markers (Richards et al., 1991). These are extremely useful in cases where an affected individual is already known and the mutant haplotype has been established. We routinely employ the (CA) markers DXS548 and AC1/AC2 for such an investigation. As these tests provide a definite genetic typing, they are very useful in cases of prenatal diagnosis.

The development of PCR based CGG amplification tests (Fu et al., 1991; Pergolizzi et al., 1992) has complemented the more time-consuming Southern blot techniques, although in no means has replaced it as a complete test. Problems with the amplification of the full mutation and possible bias in the amplification of smaller alleles in some samples requires verification of some tests by the Southern blot methods. For the determination of normal and small allele lengths, these PCR reactions are the optimum test.

MOSAICISM

The nature of the amplification of the CGG repeat element leads to mosaicism between individual cells. This is normally resolved on Southern blot analysis of a leukocyte sample as a heterogeneous smear of fragments. Individuals have been identified, however, where the mosaicism is between mutation classes, i.e., a sample gives a mixture of pre- and full mutation (Knight et al., 1992). Consideration must be given to the possibility of the extreme mosaic showing tissue variation throughout the body. This scenario could give rise to apparent premutation individuals carrying the full mutation in the germline. It may also explain apparent affected premutation individuals who presumably carry the full mutation in a tissue critical for the development of the clinical phenotype.

We have examined tissues of an affected fetus for the degree of mosaicism of mutation (Wohrle et al., 1992a). All tissues examined exhibited a high degree of CGG amplification and methylation, although the patterns of each was variable between tissues. The degree of fragile X expression in the different tissues also varied with a slight correlation with the degree of methylation. This indicates that the degree of methylation of the mutant gene may influence cytogenetic expression of the fragile site.

THE IDENTIFICATION OF FRAXE

Cytogenetic analysis for the expression of the fragile X site has been used as the diagnostic criteria in the fragile X syndrome. By definition, all affected males exhibit the fragile site in their lymphocytes. Upon the rescreening fragile X families by direct DNA analysis, it has become clear that some families do not have amplification mutations at the FMR-1 locus (Nakahori et al., 1991). In order to investigate this phenomenon, the site of chromosome fragility in these individuals has been examined directly by Fluorescence *In Situ* Hybridization (FISH). This has led to the identification of the site of fragility in some of these individuals as being further distal from FRAXA (Flynn et al., submitted). This new fragile site has been called FRAXE (Sutherland & Baker, 1992) and its precise role in leading to mental impairment is not yet clear. It lies approximately 500Kb distal to FRAXA and is flanked by the marker loci 141R (Hirst et al., 1991c) and VK21. In situations where fragile X positive, insertion negative individuals are found, an investigation by FISH is the diagnostic test of choice to rule out involvement with FRAXA.

THE MARTIN-BELL PHENOTYPE WITH NO FRAGILE SITE EXPRESSION

Investigation of a fragile X negative, Martin-Bell phenotype male identified a *de novo* microdeletion of less than 250Kb across the FMR-1 gene region (Wohrle et al., 1992b). The proband is the first child of nonconsanguineous healthy parents. His global development, including speech, was delayed and at the age of 9 he had an IQ of 55. Physical examination revealed the typical features of the fragile X syndrome. The deletion extends distal from the (CA) repeat marker DXS548 to remove the first four FMR-1 exons. Thus, the CGG trinucleotide repeat motif and the FMR-1 CpG island/promotor region is absent in this individual.

This case provides further evidence that the loss of function of the FMR-1 gene causes mental retardation and the clinical phenotype of the fragile X syndrome. We would predict that other individuals presenting with the clinical phenotype of the fragile X syndrome, in whom the stepwise amplification of the CGG repeat is absent, will be identified with deletions and perhaps point mutations. Further analysis of other such individuals is essential both to increase our knowledge of the functions of the FMR-1 gene, and to allow such individuals to enter the specialist therapies available to fragile X individuals.

SUMMARY

The identification of the FMR-1 gene, the CGG amplification events, and our subsequent understanding of many of the phenomenon of the fragile X syndrome has occurred in a very short space of time. As such, we are very much entering a phase of verification of test procedures and clarification of their interpretation. In future years, tests will become more refined and sensitive, and our interpretation of them will become more complete allowing more accurate predictive diagnosis.

ACKNOWLEDGEMENTS

This work was funded by the Medical Research Council (UK) and Action Research for the Crippled Child.

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PRENATAL DIAGNOSIS OF FRAGILE X: EXPERIENCE IN 3 LABORATORIES

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SUMMARY REPORT

Prenatal diagnosis was co-presented by . Jenkins, Howard-Peebles and Shapiro. Jenkins gave a historical profile of the world experience on prenatal fragile X detection reviewing reports from the cytogenetics literature as well as recent citations on direct DNA prenatal fragile X analyses. Howard-Peebles described the experience of our 3 centers which comprise the majority of the USA experience. Prospective and retrospective cytogenetic plus direct DNA testing was also summarized. Shapiro discussed the pitfalls that have arisen or may arise in light of the new DNA technology. These presentations are detailed below in the respective co-presenter reports.

INTRODUCTION

Prenatal diagnosis of fragile X utilizing cytogenetic techniques has been available on an experimental basis since 1981 (Webb et al., 1981; Jenkins et al., 1981, 1991). Testing has been performed on amniocytes, chorionic villus cells and fetal blood. False negative males have been reported with all 3 tissue types. Amniocytes (AF) have been the tissue of choice in the United States while chorionic villus cells (CVS) were the tissue of choice in Europe and Australasia (Jenkins et al., 1991; Shapiro et al., 1991). This report will summarize the experience of 3 cytogenetic laboratories: NYS Institute for Basic Research in Developmental Disabilities, Staten Island (Jenkins), New York Medical College and Westchester County Medical Center, Valhalla, NY and Regional Medical Genetics Laboratory Thiells, NY (Shapiro) and Genetics & IVF Institute, Fairfax, VA (Howard-Peebles). This experience includes over 600 prenatal specimens.

PRENATAL CYTOGENETIC EXPERIENCE

The specimens received by the laboratories came from multiple sources and from pregnancies with varying risks for inheriting the fragile X. Some families had not been documented to be fragile X positive. Table I summarizes the number of cases and family type received since 1981 (1988 for Howard-Peebles). Table II breaks down the case load by specimen type as well as indicating the number of fragile X positive cases found for each tissue type.

Table I
Prenatal Specimens Tested for Fragile X with and without Previous Fragile X Diagnosis

Center	Total Cases	Fragile X (+)	Fragile X (nt)
Jenkins	247	203	44
Shapiro	358	218	140
Howard-Peebles	56	44	12

nt = not tested

Table II
Prenatal Fragile X Testing by Specimen Type

		Procedure			
Center	Total Cases	AF	(+)	CVS	(+)
Jenkins	247	194	(19)	53	(13)
Shapiro	358	300	(29)*	33	(4)*
Howard-Peebles	56	41	(7)	15	(5)

*The 25 cases referred after a fragile X cell was detected in culture are not included.

PRENATAL EXPERIENCE: CYTOGENETIC AND DIRECT DNA ANALYSES

As documented elsewhere in these proceedings, direct DNA analysis became possible in June, 1991. This has allowed specimens to be evaluated using both cytogenetic and DNA procedures as summarized in Table III. Probe StB12.3, provided by Mandel (INSERM, Strasbourg, France), was used by all 3 laboratories; PCR testing (Pergolizzi et al., 1992) was utilized in some cases by Jenkins.

Retrospective direct DNA analysis has been possible in some cases with stored specimens or clinical access to the families (Table IV). In all cases in Table IV, the direct DNA result agreed with the previous cytogenetic result.

Table III
Fragile X Prenatal Testing using Cytogenetic and Direct DNA Testing

		Procedure			
Center	Total Cases	AF	(+)	CVS	(+)
Jenkins	21	12	(4)	9	(3)
Shapiro	6	2	(1)	4	(1)
Howard-Peebles	11	3	(2)	8	(4)

Table IV
Retrospective Prenatal Fragile X Testing

		Procedure			
Center	Total Cases	AF	(+)	CVS	(+)
Jenkins	9	6	(6)	3	(3)
Howard-Peebles	12	7	(4)	5	(2)

ACKNOWLEDGEMENTS

The author wishes to thank Drs. Shapiro and Jenkins and their colleagues for sharing their data. A special thank you to Dr. Anne Maddalena and staff for the DNA testing on Genetics & IVF Institute patients and to Drs. Susan H. Black, David P. Bick and Joseph D. Schulman for clinical support. Drs. Nancy Carpenter, Charles Schwartz and Jack Tarlton have helped with one or more of the author's cases. Thanks also to many colleagues who referred specimens to the author. Technical support was provided by Sharon J. Mosel, Denise Wilson and Pamela J.

Baumann.

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PRENATAL DETECTION OF FRAGILE X: EXPERIENCE OF 10 YEARS INCLUDING INTRODUCTION OF MOLECULAR TESTING

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INTRODUCTION

At least 114 cases of prenatal fragile X detection have been reported in the literature since 1981, from an estimated 1,000 specimens obtained at amniocentesis (AF), chorionic villus (CVS), and peripheral umbilical blood sampling (PUBS). These reports are documented in Table I and were summarized in Howard-Peebles, Jenkins and Shapiro co-presentations on "Prenatal Diagnosis" at the Third International Fragile X Conference, by Jenkins. The dates indicate the first time a report was received from a given laboratory. The actual citations are either in this paper or are listed in a previous report (Jenkins et al., 1991).

As of July, 1992, our center has screened over 250 cases of which 34 (19 AF and 15 CVS) were positive for FRAXA. Many (206) of these cases were from families with previous fragile X documentation. One of these was a false negative likely due to the type of fragile X induction system used. Because there have been at least 13 false negative findings reported cytogenetically, some of the factors that may contribute to their prevention will now be mentioned.

FACTORS THAT MAY PREVENT FALSE NEGATIVE DIAGNOSIS

Most of these factors are documented in a chapter in press by Jenkins, Shapiro and Brown (1992). One important factor to help prevent false negatives cytogenetically is the use of multiple fragile X induction systems. At times, one system may not detect the fragile X chromosome at all while another might be very effective. We and others have shown in the past the number of cells analyzed is critical if the frequency is very low. In a fixed suspension of cells, the distribution of the cells exhibiting the marker chromosome would be homogeneous or random, so at least 50 cells would have to be analyzed in order to identify a frequency of $\geq 6\%$ fragile X positive cells at a 95% level of confidence. In addition the number of flasks analyzed as well as the type of culture medium used may help to prevent false negative results.

Table I
Fragile X Chromosome Identification in Amniotic Fluid (AF), Chorionic Villus (CVS) and Peripheral Umbilical Blood Samples (PUBS) reported by 17 Laboratories or Groups of Investigators

Lab/Report	AF	CVS	PUBS	Total	False -	False +
Jenkins et al. (1981, Dec. 5th) *	15	6	0	21	1 AF(f)	0
T. Webb et al. (1981, Dec. 19/26)*	0	0	11	11	1 CVS(m) 1PUBS (m)	0
Shapiro et al. (1982)	19	2	2	23	2AF(m) 1AF(f) 3CVS(m)	0
Schmidt et al. (1982)	6	0	0	6	0	0
Tejada et al. (1983)	1	0	0	1	0	0
Wilson, Marchese (1984)	1	0	0	1	0	0
Hogge et al. (1984)	1	0	0	1	0	0
Tommerup et al. (1985)	1	1	0	2	0	1AF(m)
von Koskull et al. (1985)	5	5	0	10	0	0
Rocchi et al (1985)	1	0	2	3	1AF(m)	0
G. Webb et al. (1986)	4	0	0	4	0	0
Howard-Peebles (1986)	5	3	0	8	1AF(m)	0
Sutherland et al. (1987)	2	2	0	4	1CVS(m) [incon]	0
Purvis-Smith et al. (1988)	4	6	0	10	1AF(f)	0
McKinley et al. (1988)	0	0	3	3	0	0
Kennerknecht et al. (1991)	0	3	0	3	1CVS(f)	0
Devillard et al. (1992)	3	0	0	3	0	0
Total No.	68	28	18	114	7AF 5CVS 1PUBS	1AF
%	59.6	24.6	15.8	100.0		
R** Value	68/75 90.7	28/33 84.8	18/19 94.7	89.8	--	99.1

* listed chronologically according to publication date, e.g., Jenkins et al's. letter was published on December 5th, 1981 and Webb et al's. was published on December 19/26, 1981; some groups may have more recent citations, reported in either Jenkins et al. (1991) or in the references for this report

** R or reliability value as defined in Jenkins et al. (1991) and further discussed in Jenkins, Shapiro, and Brown (1992)

The use of positive control cultures such as long-term lymphoblast or amniocyte cultures derived from pre-identified fragile X individuals has been helpful to assure the chemicals used to visualize the fragile site are prepared correctly. Further, some laboratories rely upon one or a combination of fragile sites other than fra(X)(q27.3) to indicate the fragile X induction system is working properly. An awareness of fragile sites located very close to the site at Xq27.3 is very important. Such fragile sites may be located very close to or even perhaps at the same location as the one associated with the fragile X syndrome (Sutherland and Baker, 1990; Zaslov and Brown, 1991; Dennis et al., 1992; Jenkins et al., 1992; Oberle et al., 1992)

DNA linkage studies have, in some cases, increased the reliability of prenatal diagnosis to greater than 99%. But such studies are often not practical, given the time it takes to study the entire family and often the pregnant woman waits until virtually the last minute to ask for help. But most importantly, direct DNA and PCR studies of the fragile X mutation, FMR-1, have revolutionized testing for the mutation both pre- and postnatally. Thus far, 15 cases have been reported, detected both retrospectively and prospectively in CVS, AF and PUBS specimens cultures, as shown in Table II. With the application of direct DNA testing, a protocol that is not only more rapid and reliable, but allows prediction of the degree of affectedness, the use of prenatal fragile X testing is likely to increase many times.

Table II
Direct DNA Prenatal Detection of the FMR-1 Gene

Lab/Report	AF	CVS	PUBS	Total	False -	False +
Hirst et al., 1991	0	1	0	1	0	0
Dobkin et al., 1991	1	0	0	1	0	0
Rousseau et al., 1991, 1992	1	10	1	12	0	0
Sutherland et al., 1991	0	1	0	1	0	0
Total	2	12	1	15	0	

As validation studies continue, it is expected that false negative and positive diagnoses (Oberle et al., 1992; Romain and Chapman, 1992) will be very rare thereby increasing the reliability of prenatal fragile X diagnosis to nearly 100%.

EXPERIENCE WITH DIRECT DNA AND PCR TESTING

From June, 1991 to present, we have been using a combination of PCR, direct DNA

(StB12.3) (for methods see Brown et al., these proceedings) and cytogenetic validation. Thus far, 25 AF or CVS specimens have been screened. As a control for PCR testing, 12 retrospective samples (AF, CVS or abortus specimens) were analyzed by PCR and found in agreement with previous negative or positive cytogenetic and DNA linkage results. Sexing the specimens via PCR analysis and a rapid PCR testing protocol has allowed us to obtain results within three days of receiving the CVS.

Thus far the fragile X mutation, FMR-1, has been identified in 4 AF specimens, 3 females and 1 transmitting male, and 5 CVS specimens, 3 females and 2 males. One of the negative CVS specimens was questionable cytogenetically and was found to be clearly negative using the molecular protocols. One of the AF specimens exhibited a very low frequency of fragile X positive cells (3 in 400) but was confirmed to be positive via molecular testing. All other results to date were in agreement indicating that PCR and direct DNA testing are thus far reliable, efficient and correctly predict the phenotype. For the present, we suggest a preliminary PCR analysis in combination with selective follow-up by direct DNA and cytogenetic testing, for purposes of both validation and appropriate clarification.

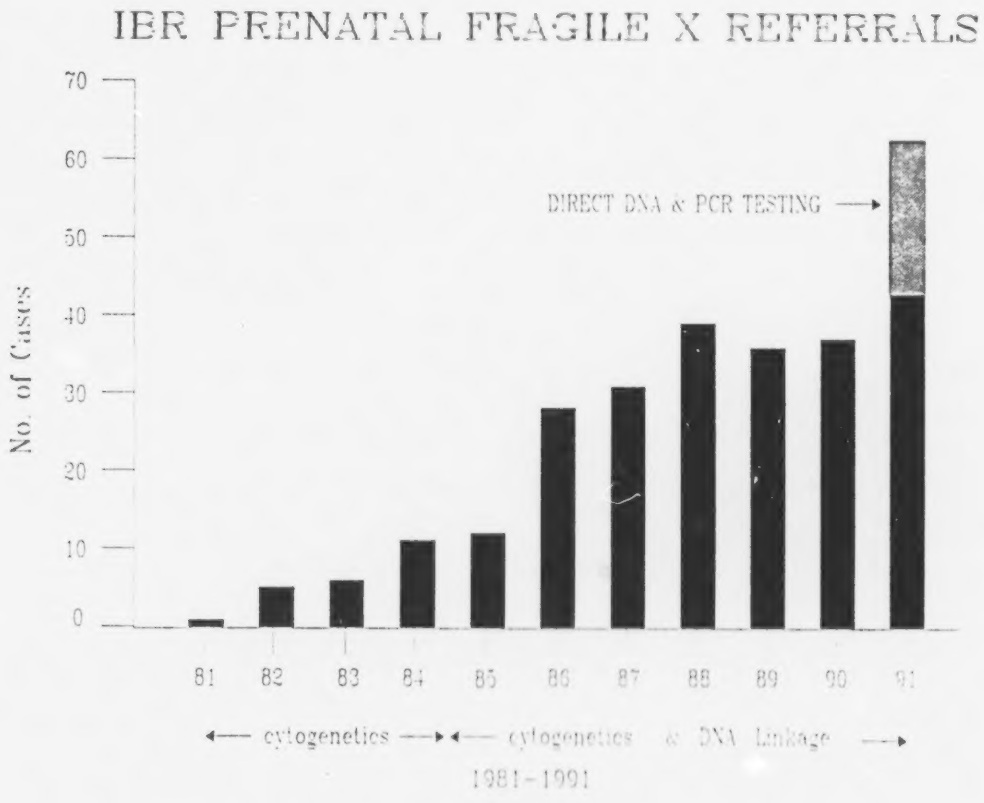

Figure 1

The use of molecular technology for prenatal diagnosis of the fragile X mutation, FMR-1, should lead to increased utilization of prenatal diagnosis and the primary prevention of the disorder. Figure 1 indicates the increase in referrals of both AF and CVS specimens to our laboratories. During 1991, a very significant increase of pregnant women were referred both for PCR and direct DNA testing of themselves to determine their carrier status, as well as follow-up prenatal diagnosis, when appropriate. The attached part of the bar graph over 1991 indicates the increase in referrals experienced during 1991 (and continuing in 1992).

ACKNOWLEDGEMENTS

We thank Dr. Charles Schwartz for providing information on the direct DNA test that was performed in his laboratory on one of the fragile X positive specimens reported here. We also thank Drs. James Ray and Maureen Sanz for their constructive criticism in the preparation of the manuscript. We also acknowledge the assistance of Micole Cohen in relation to library work necessary for the preparation of this paper. Thanks are also due Mrs. Leslie Woolsey for secretarial assistance and her contribution to both word-processing and art work. This study was supported in part by funds from the New York State Office of Mental Retardation and Developmental Disabilities, and in part by grant MCJ360587 from the Maternal and Child Health Program (Title V, Social Security Act), Health Resources and Service Administration, Department of Health and Human Services.

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PITFALLS IN PRENATAL DIAGNOSIS OF THE FRAGILE X SYNDROME

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There are a number of potential pitfalls and technical problems related to direct DNA testing for the fragile X (fra X) gene in amniotic fluid cells or chorionic villus samples (CVS) which can be better appreciated and clarified by the use of concurrent fra X cytogenetic analysis. In addition, the use of concurrent fra X cytogenetic analysis and direct DNA testing may enable a more precise interpretation under certain circumstances

POTENTIAL TECHNICAL ARTIFACTS

A number of technical artifacts have been described (Rousseau et al., 1992) which can reduce the reliability and sensitivity of the direct DNA analysis of fra X mutations:

A) Incomplete digestion - because fra X mutations can result in almost any pattern of several or multiple bands of increased size, a partial digest or a band due to plasmid contamination could be mistaken for a mutated fragment. Negative fra X chromosome analysis would be helpful in appreciating this artifact.

B) Loading of undegraded DNA - loading an incorrect amount of undegraded DNA can result in an unfocused smear caused by heterogeneous mutations in the male being missed while the normal band is absent. In a female, an unfocused smear may represent an even more difficult problem because the fragment from the normal X is present. A positive concurrent fra X cytogenetic result would call attention to these artifacts and result in the detection and lead to steps by which they can be controlled.

C) Sample loading - sample overloading may cause migration differences which can be mistaken for abnormal fragments or tight doublets can be missed if the bands are too broad. In this situation, concurrent fra X chromosome analyses would enable detection and correction of these artifacts.

Mosaicism for the Size of the Unstable DNA Fragments (number of CGG repeats)

The presence of an unfocused smear which goes undetected in association with a normal size fragment or one in the premutation range can result in a false prediction and an inaccurate interpretation for prenatal diagnosis. A concurrent positive fra X chromosome result would enable a correct interpretation and detection of the technical problems. Mosaics can also lead to an incorrect prenatal diagnostic result when a PCR-based assay is utilized. It has been shown that the number of CGG repeats can be determined accurately for premutations by PCR-based assay (Fu et al., 1991). Although the PCR-based assay is more rapid than the Southern blot, the lack of detection of full mutations is a potential problem, especially in mosaics, because of selective detection of premutation. This would result in an incorrect prenatal diagnosis which could be avoided by concurrent use of fra X chromosome analysis with a positive result.

Heterogeneity of Mutations

The number of CGG repeats and subsequent size of the unstable DNA fragment can be heterogeneous. Thus, distinguishing the normal transmitting male from the affected male and unaffected carrier female from the affected female can be difficult simply because the number of CGG repeats can overlap due to heterogeneity. The presence of a positive fra X chromosome analysis can establish whether or not a male or female fetus is affected.

Incomplete Methylation of Extra-embryonic Tissues (CVS)

Methylation of the full mutation in chorionic villi at the 8th-10th week of pregnancy is not always present (Rousseau et al., 1992; Devys et al., 1992), and X-inactivation (methylation) in the female fetus may not occur or be incomplete in CVS. Thus, interpretation of results utilizing methylation status can be difficult in CVS, and concurrent fra X cytogenetic analysis could be advantageous.

In addition to the foregoing, in prenatal diagnosis there is no available phenotype for comparison with the laboratory results in a situation where important decisions must be made. Thus, as contrasted to postnatal specimens, the use of parallel direct DNA testing and fra X chromosome analysis can be of great value in assuring accurate and reliable prenatal diagnosis results.

We have reported on over 300 amniotic fluid specimens for prenatal diagnosis and calculated the accuracy of the prenatal cytogenetic diagnosis for fragile X syndrome to be approximately 97% (Shapiro et al., 1992). These calculations take into account the *a priori* risk for the fragile X syndrome for each pregnancy. In addition, the principal investigator has never experienced a false positive, and there is only one report of a false positive result in the literature (Jenkins et al., 1991).

With time and experience, it is probable that the cut-offs necessary to distinguish normal variation, premutation and full mutation will be determined. In addition, as the pitfalls and technical artifacts are identified, it is likely that they will be eliminated and corrected by means of internal controls and appropriate technical modifications. The use of concurrent direct DNA testing and fra X chromosome analysis will enable the appreciation of these difficulties and correct interpretations in utero so that incorrect diagnoses and interpretations are not discovered *after* the pregnancy has been completed. It is possible and even probable that with increasing experience and elimination of the pitfalls and technical artifacts, fra X cytogenetic analysis will no longer be necessary; however, at this time, during the period of validation and determination of sensitivity, specificity and accuracy, it would be prudent for both methods of testing to be utilized concurrently.

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CYTOGENETICS OF THE FRAGILE X SYNDROME: PAST, PRESENT AND FUTURE

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Although known since the 19th century that there was an excess of mentally retarded males over females indicating that genes for intelligence were located on the X chromosome (Lehrke, 1974), it was not until 1943 the large pedigree documenting X-linked mental retardation (XLMR) was published by Martin and Bell. Future research workers were warned by this pedigree, as it not only contained affected females but also transmitting males! Further pedigrees documenting X-linked mental retardation were published by Renpenning et al. (1962) and by Dunn et al. (1963), but as the latter family did not present with idiopathic mental retardation it was obviously not due to the same defect. The fragile X syndrome was called the Martin-Bell-Renpenning syndrome for a short period until cytogenetic techniques differentiated between the two.

The first cytogenetic demonstration of the fragile X came in 1969 with a landmark paper by Herbert Lubs. Published in the days before the establishment of chromosome banding techniques, this paper not only first described the fragile site lying distally on a C group chromosome, but correctly assigned it to the X. The author traced the fragile chromosome through an XLMR pedigree with four affected males and two unaffected carrier females, and even performed X-inactivation studies on the female cells. The stage was then set for identification of the fragile site as definitely on the X and its use as a marker for some XLMR when chromosome banding was first described in 1971. Chromosome banding, either by fluorescent methods or utilizing trypsin and Giemsa, permitted individual identification of each one of the 24 human chromosomes.

For the next several years the diagnosis was rarely made. This apparent loss of the fragile X chromosome was due to technical improvements in the culture of lymphocytes for chromosome studies. In the early days, the medium used for cytogenetic studies in many laboratories was TC199, which contained very little folic acid. Enterprising medium makers later formulated RPMI 1640 which they rightly claimed to improve both the yield and the quality of mitoses for cytogenetics. However, this medium did contain folic acid and if employed for cytogenetic studies, the mitoses did not demonstrate fragile X or FRAXA as it is now known. Despite confirmation by Giraud et al. (1976) and by Harvey et al. (1977) that the fragile site was indeed on the X, it was left to Sutherland (1979) to make the connection and to demonstrate unequivocally that the composition of the culture medium was of vital importance for the demonstration of the fragile X and that in order to do so the medium must contain neither folic acid nor thymidine. If a rich medium such as RPMI is used for cell culture, then the fragility could be demonstrated by blocking the enzyme tetrahydrofolate reductase with the folate antagonist methotrexate (Sutherland, 1979), or by blocking thymidylate synthetase with the thymidine analogue FUdR (Glover, 1981). With the establishment of these methods for reliably demonstrating the fragile site and for precisely locating it at Xq27.3, many laboratories were able to analyze a large number of pedigrees, and the cytogenetic analysis of the fragile X syndrome was established. At this point, certain individuals from the original Martin-Bell family were re-examined and they did, indeed, carry the fragile X gene (Richards et al., 1981).

However, problems began to emerge very quickly. All the individuals who did demonstrate the fragile site appeared to be mosaics, the fragility was never visible in 100% of the mitoses and rarely found to exceed 50%. Inclusion of chemicals such as methotrexate or FUdR or even just growing cells in non-ideal conditions can also cause random damage. There is always some level of chromosome fragility, even in control cultures. Thus, there was a cut-off point below which fragility could occur in the general population. As some fragile X positive individuals, particularly unaffected carrier females, have low levels of FRAXA in their cells, this phenomenon was an insurmountable obstacle in the accurate cytogenetic diagnosis of the syndrome.

Other problems were foreshadowed in the Martin-Bell pedigree; first, about one third of the female carriers of the syndrome were themselves affected, and second, one fifth of the males who carried the gene were not. This latter group comprised the transmitting males. Affected females, like affected males, generally demonstrated FRAXA in a significant proportion of their cells and could be detected by cytogenetic methods. Transmitting males, and up to one half of the unaffected female carriers including all daughters of transmitting males, however, did not demonstrate the fragile site and were not detectable cytogenetically. In the mid nineteen-eighties, two further papers from Adelaide showed it was the balance of thymidine within the pyrimidine pool that mattered rather than its removal, as an excess could also induce FRAXA (Sutherland et al., 1985; Sutherland and Baker, 1986). As this is a much kinder induction method than either methotrexate or FUdR, the background level of chromosome damage was reduced, making slides easier to analyze. It was observed throughout this period the fragile site at Xq27.3 was one of many such fragile sites scattered throughout the karyotype. It remained, however, the only one which was associated with a clinical phenotype. Many of the other sites were ubiquitous and induced at a variety of levels by a variety of agents. Indeed, the detection of the most common fragile site, at 3p14, has been suggested as a control for the detection of FRAXA. The fragile site at Xq27.3 is designated a "rare" site as it presents in a small percentage of the population. A "common" fragile site is one which can be induced in cells from a high percentage of the population but generally at a very low level. In 1990, Sutherland and Baker showed that there was a common fragile site (FRAXD) situated at Xq27.2 and that this site could be confused with, and therefore cause the incorrect diagnosis, of FRAXA. Cytogenetic diagnosis of fragile X not only requires a high level of expertise and is laborious because it is present in only a few percent of mitoses, but can also be inaccurate.

Cytogeneticists interested in the fragile X syndrome pooled their experiences and established guidelines regarding the media used, the additives to maximize demonstration of FRAXA and the numbers of cells to be studied (Jacky et al., 1991). After the demonstration of fragile sites in fibroblasts as well as in lymphocytes (Jacky and Dill, 1980), and reports of its detection in fetal cells (Jenkins et al., 1981; Webb et al., 1981), prenatal diagnosis was done in amniocytes, fetal lymphocytes, and later, CVS samples. Reliability in induction of FRAXA in monolayer cells such as amniocytes was difficult but achieved (Jenkins et al., 1986; Jenkins et al., 1991).

The level of cytogenetic expression of the fragile X in affected males is variable. Although repeated analyses of samples from the same individual taken over a time period do show a relative degree of reproducibility, there is greater consistency of levels within families rather than between them. In general, marked variations in levels of expression exist among affected individuals and there is no correlation between the level of expression and the degree of intellectual handicap. Transmitting males, who are clinically normal but who can have affected grandchildren via daughters who are also clinically unaffected, do not generally express the fragile X at all.

Female family members present the cytogeneticist with an even greater challenge. There are four types of female carriers in fragile X families: the unaffected, both with and without demonstrable fragile X; and the intellectually impaired, again both with and without demonstrable fragile X. Between one and two thirds of the obligate carrier females have been found to be intellectually normal and have no phenotypic manifestation of the syndrome, and 90% of these are also FRAXA negative making carrier detection very inaccurate. The remainder of the normal carriers are FRAXA positive. Of the affected females, the majority do demonstrate the fragile site in a significant number of their cells, but the degree of impairment may be minimal.

Obviously cytogenetics alone did not answer many questions regarding carrier status. Molecular methods of identifying the Martin-Bell syndrome were necessary. The first report of linkage between FRAXA and a proximal X-chromosome marker, factor 9 (F9) (Camerino et al., 1983) came in 1983. Despite the lack of crossing over between the two markers in this first family, a high level of recombination was soon reported. Finding of a highly informative marker on the distal side, St14, at DXS52, (Oberle et al., 1986), permitted the use of flanking markers as an aid in the diagnosis of the fragile X syndrome, but the high degree of recombination led to the region being designated a "hot spot." Gradually, closer flanking markers permitted more accurate identification of individuals who had inherited the Martin-Bell gene, which taken together with cytogenetic methods, reached an accuracy greater than 1%.

In 1991, the now classic series of papers identified the presence of an insert or amplification in one of the exons of the FMR-1 gene which is almost completely associated with the presence of the fragile X (should we now rename it?) or Martin-Bell syndrome (Vincent et al., 1991; Bell et al., 1991; Yu et al., 1991; Oberle et al., 1991; Verkerk et al., 1991). The insert can be directly visualized in both males and females. The transmitting males and the unaffected females both show a small but still detectable amplification which has been termed the premutation. Affected individuals both male and female, and some unaffected female carriers, have a larger amplification accompanied by methylation of an upstream CpG site which has been termed the full mutation. This direct molecular method is accurate, fast and inexpensive. What part, if any, will cytogenetics play in the future diagnosis of the fragile X syndrome?

While the molecular methods are being evaluated, cytogenetic studies must be used in parallel with them. The reasons for any discrepancies must be determined and documented. There is a protocol to do so from pooled data from participating laboratories. Also, laboratories with many referrals for cytogenetic studies for developmental delay or mental handicap have detected many other cytogenetic abnormalities in addition to FRAXA. These other cytogenetic diagnoses will be missed if such referrals are studied by molecular methods only. It is proposed, therefore, that all probands be studied both by molecular and cytogenetic methods. If an insert/amplification is detected, and fragile X syndrome diagnosed, then the family can be followed up using molecular methods only. A combination of cytogenetic and molecular methods may also aid in the diagnosis of those families who manifest the Martin-Bell syndrome as a consequence of other mutations such as deletions or point mutations in the gene. Particular interest has been aroused by the finding of an alternative fragile site FRAXE which lies just distal to FRAXA and is present in some families who do not demonstrate the insert.

Prenatal diagnosis of the syndrome should involve both cytogenetic and molecular methods. Only when all the problems, such as the apparent lack of methylation of the CpG island in CVS samples or consistency in the interpretation of mosaic patterns, have been overcome will it be feasible to decide if both methods are needed to maximize reliability or whether direct molecular methods can stand alone.

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THE OCCURRENCE OF CHROMOSOMAL ABNORMALITIES IN SPECIMENS REFERRED FOR FRAGILE X ANALYSIS

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INTRODUCTION

In the time period from 1981 to 1992, our laboratory has identified a significant number of fragile X cases. In preparation for our presentation on "The Role of Cytogenetics" at the 1992 Third International Fragile X Conference held in Snowmass, Colorado, June 16-20, we reviewed our specimen referrals from 1987 to 1992 to determine the frequency of chromosome abnormalities we detected while testing for the presence of the fragile X chromosome. A total of 1,956 cases referred for fragile X studies and/or standard karyotype analysis were retrospectively reviewed. The results indicated that chromosomal abnormalities, excluding trisomy 21, were present in 4.6% of samples representing 38% of the fragile X abnormality rate. These findings are the subject of this report.

METHODS

Whole blood cultures tested for fra (X) (q27.3) were incubated in either a continuous folate deficient system or were exposed to 5-fluorodeoxyuridine (FUdR) according to procedures described elsewhere (Jenkins et al., 1990). If a specimen was not monitored for the fragile X chromosome, complete medium containing RPMI-1640 (GIBCO) was utilized together with non-dialyzed fetal bovine serum without exposure to FUdR (Jenkins et al., 1991). All specimens were analyzed for chromosome abnormalities by microscopically analyzing 20 cells and printing two or more G-banded karyotypes using a Zeiss photoscope coupled to a semi-automated chromosome analyzer and laser printer (Perceptive Scientific Instruments, Inc.). Most of the analyses were carried out according to the guidelines recommended at the Fourth International Workshop on Fragile X and X-linked Mental Retardation in 1989 (Jacky et al., 1991), while the remainder included 50-100 cells per specimen for fragile X chromosome analysis alone. Chromosome abnormalities here refer to aneuploidy as well as unbalanced structural chromosome rearrangements.

RESULTS

Figure 1 shows the general distribution of results from all whole blood referrals to the laboratory whether or not they were analyzed for fra(X)(q27.3). Of the total specimens ascertained for either fragile X or standard karyotype analysis without fragile X study, a significant number of chromosome abnormalities was observed in both types of studies.

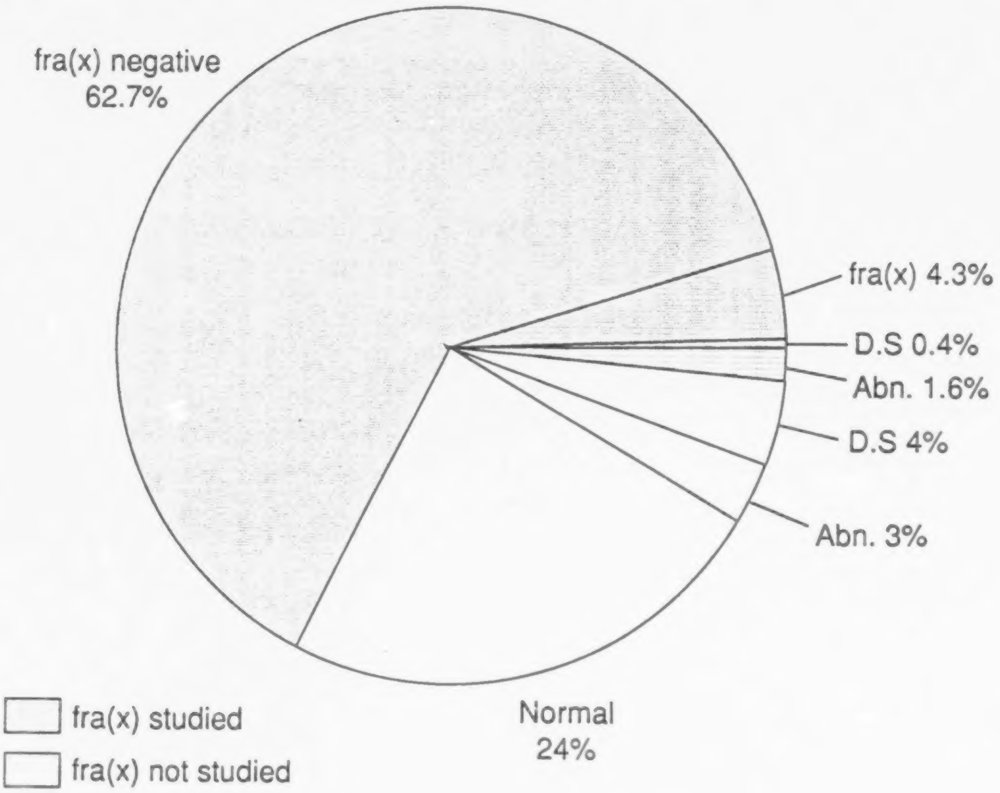

Figure 1. Distribution of results from all whole blood referrals whether or not they were analyzed for fra(X)(q27.3).

Fig. 2 shows that the fragile X chromosome was identified in 6.3% of the specimens that were screened for fra(X)(q27.3), while abnormalities other than DS (0.5%) were seen in 2.4% of the cases.

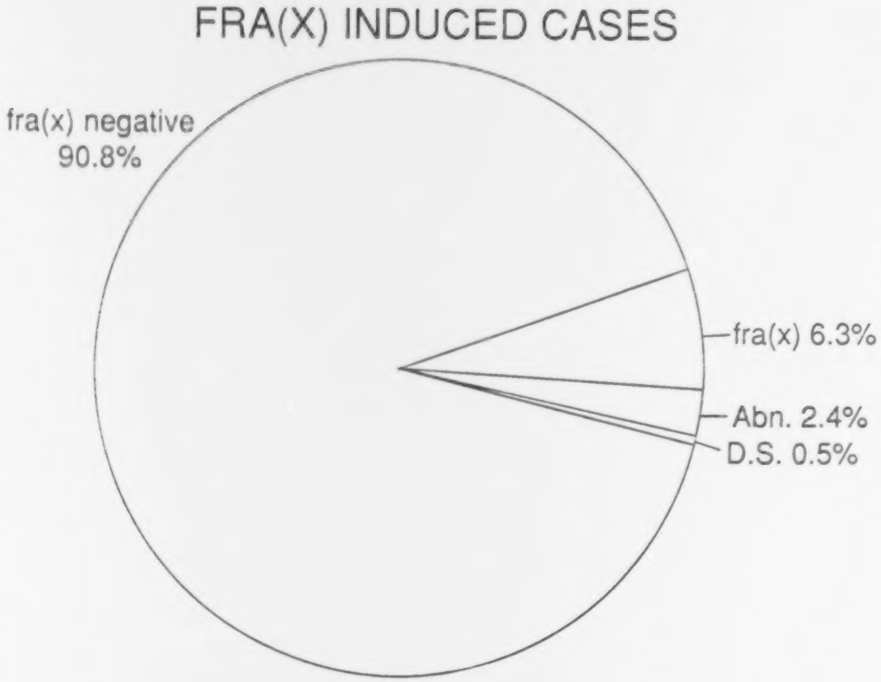

Figure 2. Distribution of results from whole blood referrals for fragile X analysis.

The group that was studied without fragile X chromosome analysis showed 9.7% (Fig. 3) with various chromosome abnormalities and 13.0% with DS for a total of 22.7%.

The distribution of these results with the actual number of cases is summarized in Table 1. The distribution of results when the specimens are separated into two groups: (1) 1349 specimens analyzed for both the fragile X chromosome and the standard karyotype; (2) 607 specimens analyzed for the standard karyotype only.

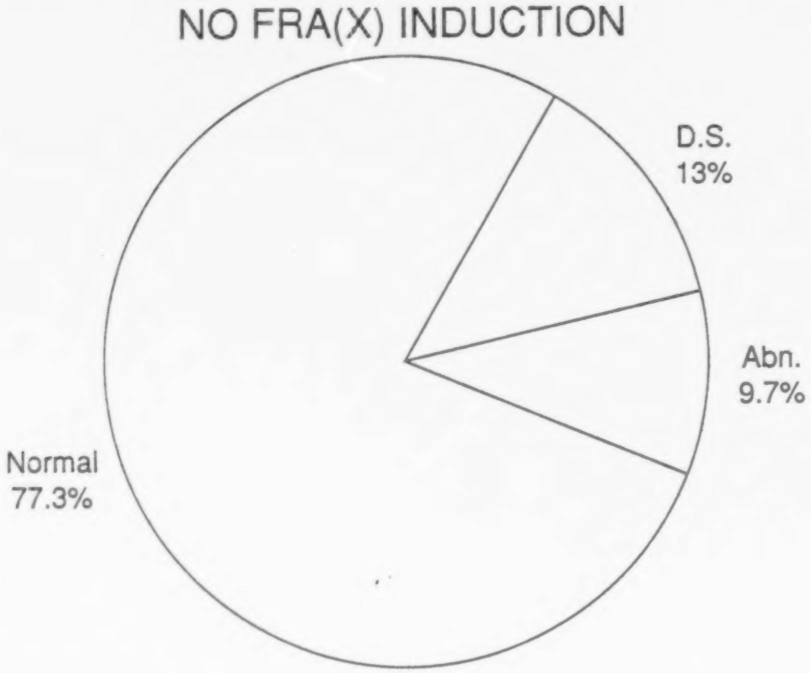

Figure 3 Distribution of results from whole blood referrals made for standard chromosome studies, without fragile X screening.

Table 1
Distribution of Results from Specimens
Studied for fra X and for Standard Karyotyping Only

Group	Total	Normal	fra X	Abn.	+21	total
1 fra (X)	1349 (100)	1225 (90.8)	85 (6.3)	32 (2.4)	7 (0.5)	39 (2.9)
2 standard	607 (100)	469 (77.3)	--	59 (9.7)	79 (13.0)	138 (22.7)
Total	1956	1694	85	91	86	177

As indicated in Figure 1, when trisomy 21 is excluded the percentage identified was about 1/4 of that observed in Group 2.

DISCUSSION

From the observations made retrospectively in Figs. 1-3 and Table 1, it is clear that including standard karyotype analysis was worthwhile when screening for the fragile X chromosome. Otherwise, a number of chromosome abnormalities would not have been identified, even though referrals were made primarily for the possibility of the fragile X mutation. These results are in agreement with another report (Dewald et al., 1992) that tested the guidelines for cytogenetic fragile X testing recommended by Jacky et al. (1991). They also confirm and extend two previous reports on our screening programs in New York (Nolin et al., 1991, 1992), as well as Turner's et al. (1992) recent report on fragile X population screening.

However, in view of the recent advances in molecular genetics as indicated in the articles by Brown et al. and Oostra et al. in these proceedings and since direct DNA and PCR analysis can now be performed more cheaply and efficiently, chromosome analysis for the fragile X mutation may no longer be the method of choice. It is realized that for some institutions, accessibility to molecular testing may be a problem for the short term. For detecting the full mutation, cytogenetic testing is still valid. Also, cytogenetics still will be required for clarification in cases where molecular analyses have led to discrepant results.

It is estimated that a skilled cytogenetic technologist can process, completely, approximately 5 cases per week, when both fragile X identification and standard karyotype analysis are involved. On the other hand, it has also been estimated that a trained technologist in molecular genetics may process 100 specimens or more per week for fragile X identification suggesting an increase in productivity of at least 20 times!

The workshop discussion on the role of cytogenetics in fragile X syndrome diagnosis held at the Third International Fragile X Conference recommended that for the next year, some laboratories participate in a validation study of molecular fragile X identification by screening all cases referred for fragile X by both molecular and cytogenetic technology. Where laboratories felt they could not afford the resources to screen all samples, it was recommended that all positive and/or questionable samples be screened for fragile X with both cytogenetic and molecular technology.

Our current protocol is to screen all fragile X referrals using PCR analysis with selective direct DNA and cytogenetic follow-up. This will apply to both pre- and postnatal specimens. Individuals who are negative on PCR screening will be carefully re-evaluated relative to possible etiology, and where there is multi-system involvement including mental impairment of unknown etiology, cytogenetic follow-up, standard karyotype, in a folate deficient environment will be recommended. In this manner, high expressing fra X individuals will be picked up, in cases where PCR analysis was not performed. In these cases, only 20 cells will be analyzed so that the cytogenetic laboratory will be performing virtually no extra work in considering a fragile X etiology. Since there is a variable number of positive cells from one individual to the next and at times even between cultures, it is anticipated that only those cultures with fra X frequencies greater than about 14% will be identified at a 95% level of confidence (Hsu, 1986).

ACKNOWLEDGEMENTS

We thank Drs. James Ray and Maureen Sanz for their constructive criticism in the preparation of the manuscript. Thanks are also due Mrs. Leslie Woolsey for secretarial assistance. This work was supported in part by funds from the New York State Office of Mental Retardation and Developmental Disabilities, and in part by grant MCJ360587 from the Maternal and Child Health Program (Title V, Social Security Act), Health Resources and Service Administration, Department of Health and Human Services.

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PREVALENCE OF CHROMOSOMAL ABNORMALITIES IN A POPULATION REFERRED FOR FRAGILE X TESTING: THE CHILDREN'S HOSPITAL, DENVER EXPERIENCE 1983-1991.

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Since 1983, at the Children's Hospital, Denver, cytogenetic fragile X assessment has been a standard test for individuals presenting with mental retardation of unknown etiology. The referral population has included patients followed in outpatient clinics of the Child Development Unit, Genetics, and Neurology. The number of cases studied in this 8 year period is 3880. In this group, 455 individuals with chromosomal abnormalities have been identified (11.7%). There were 291 cases (7.5%) which were positive for fra(X)(q27.3). These included probands and family members. Approximately two-thirds were males. Two fragile X positive individuals were also balanced translocation carriers. Numeric abnormalities were observed in 53 cases (1.4%) and 47% of these were sex chromosome abnormalities, including 14 with 46, XXY, one of whom was also fragile X-positive. Eight cases with supernumerary markers were identified. Structural abnormalities were found in 111 individuals (3%), 38 of which were deletions. Of interest, 7 children were found to have del 17p, the microdeletion associated with Smith-Magenis syndrome. Phenotypic features that overlap the Fragile X Syndrome and Smith-Magenis syndrome include mild to moderate mental retardation, behavioral problems and hyperactivity, prognathism and large or abnormal ears. The cytogenetic preparations obtained from standard fragile X preparations were of a quality that allowed detection of small deletions and inversions. The frequency of cytogenetic abnormalities in this fragile X referral population demonstrates the value of including chromosomal assessment as part of the evaluation of individuals with some degree of mental impairment including severe learning disabilities, of unknown etiology.

SEQUENCES AROUND THE FRAGILE X REGION REGULATE ACTIVITY OF THE DISTAL PART OF THE X CHROMOSOME IN FEMALES

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We have identified two girls with interstitial deletions involving the fragile X region and the flanking sequences between DXS 297 and IDS. Both patients are mentally handicapped and one is additionally affected with Hunter syndrome. In both of them, the deleted X chromosome is preferentially active in fibroblasts and in lymphocytes, as determined by cytogenetic methods, by the analysis of DNA methylation with the probe M27b (DXS 255), and by the analysis of transcription at the IDS locus using reverse transcriptase - PCR protocol. This abnormal inactivation pattern is unique to our patients because in other females with deletions it is always the deleted X that is inactive, or otherwise the maternal or the paternal X chromosome is inactivated at random in every female cell. This suggests that some sequences around the fragile X region control inactivation of the X chromosome. When these sequences are missing the cells bearing the deleted X as inactive, and the norm as active are functionally abnormal and are eliminated by cell selection. Either the deleted X chromosome cannot be properly inactivated, or the normal X is not fully active in these cells. We can now verify these two possibilities because we have succeeded in isolating these rare lymphoblasts (~5%) that persist in one of our patients despite the negative selection. These cells are now being analyzed with methyl-sensitive probes from the distal part of the long arm of the X chromosome. So far, G6PD probe has been used and it detected only unmethylated Hpa II fragments that are characteristic of the active X chromosome. This indicates that genes distal to the deletion may fail to be inactivated, and because they are present in two active copies they promote strong negative cell selection. Therefore, it appears that the role of sequences around the fragile X region is to pass the inactivation signal to more distal segments of the X chromosome. Once the function and the location of these sequences is better characterized it may help to understand the event(s) that underlie the conversion of premutation to full mutation, which is thought to occur in oocytes of some of the women who are carries of the fragile X syndrome.

X INACTIVATION IN FRAGILE X SYNDROME HETEROZYGOTES: METHODOLOGY AND CORRELATIONS WITH IQ

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Expression of phenotype, including some degree of mental impairment, in about a third of fragile X syndrome heterozygotes is one of the unusual features of this X-linked disorder. The amplification size of the CGG repeat in the FMR-1 gene in women with the full mutation does not fully account for the degree of mental impairment, nor does the frequency of fragile X expression. Non-random or skewed inactivation pattern of the X chromosome carrying the FMR-1 mutation could account for the variability of phenotype in females. The detection of fragile sites in lymphocytes cultured under conditions that allow distinction between the early replicating, active and the late replicating, inactive X chromosome is problematic: bromodeoxyuridine (BrdU), the thymidine analogue used to visualize replication status, also inhibits fragile X expression.

We have confirmed studies by others showing that the time of BrdU incorporation, early vs. late in the S/G2 phase of the cell cycle, influences the rations of active fragile Xs [A-fra X] vs. inactive fragile Xs [I-fra X].

A group of 14 fragile X heterozygotes whose fragile site expression was >2% (range 4-33%) were studied to determine the inactive status of the fra X. In these females, a higher proportion of A-fra X is observed when BrdU is available during late S and G2. We postulate that BrdU can be incorporated more readily into the late replicating, inactive X, as well as within late replicating regions of the active X. Additionally, it would be available for incorporation through the DNA repair mechanisms in G2. When BrdU is available only during early S, a higher proportion of I-fra X is observed. Thus, an early pulse BrdU inhibits the expression of the fragile site when the mutation is on the early replicating (active) X.

While the methodology affects the ratios of A-fra X to I-fra X, it does not affect the patterns of inactivation among females when compared to another variable such as mental status. When the frequencies of A-fra X were compared with IQ, a statistically significant inverse correlation between IQ and the proportion of A-fra X was observed when the early pulse BrdU was used. IQ scores in the range of mentally retarded (N=1), borderline (N=8) and normal (N=5) are represented in this study. Preliminary FMR-1 data available for 8 of the 14 females using Ox1.9 and polymerase chain reaction (PCR) to observe CGG repeat copy number suggest that all have at least CGG repeat copy numbers of between 300-1000 bp (P2, Snow et al., 1991), at the high end of premutation or low end of full mutation. A skewed pattern of inactivation of the mutated X appears to contribute to the variation in degree of mental impairment among women with similar FMR-1 mutations.

CARRIER TESTING OF PERSONS WITH SUGGESTIVE FAMILY HISTORIES CONFIRMS A HIGH FREQUENCY OF FRAGILE X

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During routine prenatal genetic counseling, maternal family histories including one or more relatives with mental retardation or social/educational problems are encountered. Where fragile X is a possible diagnosis, we have attempted to determine if the consultand is a carrier by direct testing of her DNA, whether or not the proband is available for testing. The probe StB12.3, provided by J. L. Mandel, is used with EagI/EcoRI digestions. Negative results in females are confirmed by cytogenetic testing, because of the possibility of faint, diffuse smears. Small insertions may require additional family studies for interpretation.

In a period of three months, we identified 22 at-risk females who elected to have fragile X screening on themselves, and 6 who were able to obtain samples first from an affected son or brother. Among the 22 consultands (whose prior probabilities of sharing an X linked allele with the affected relative ranged from 1 to under 0.25), one carrier of a fragile X premutation was found. In the 6 probands with previously undefined MR or LD, 2 were confirmed to have fragile X. Thus, 3 families were ascertained among 28 with broadly defined risk of fragile X, even though many of the initial tests could not be performed on the probands or obligate carriers themselves. Altogether, 2 carrier consultands were identified among the 28.

This survey is continuing and will provide a large sample of the general American population reporting a close or distant relative with undefined mental impairment. It highlights the effectiveness of screening a broadly defined at-risk population for fragile X carrier status.

PREIMPLANTATION GENETIC SCREENING: AN OPTION FOR FAMILIES AT RISK FOR TRANSMISSION OF THE FRAGILE X CHROMOSOME

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Susan H. Black, MD, Edward F. Fugger, PhD, Frances T. Palmer, Gary L. Harton,
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The goal of preimplantation genetics is to allow parents who carry genetic diseases such as fragile X the opportunity to have unaffected children of their own. The method involves molecular genetic screening of *in vitro* fertilized embryos, followed by implantation of those free of the disorder. Diagnosis involves biopsy of a small number of cells taken from the early embryo, followed by amplification of target DNA sequences by the polymerase chain reaction. For preimplantation diagnosis, it would be desirable to identify and eliminate all embryos bearing fragile X chromosomes, including those that carry either full-mutations (large numbers of CGG repeats) or premutations (moderate numbers of CGG repeats), since (a) the syndrome can affect either males or females, and (b) chromosomes bearing premutations can undergo additional mutational events leading to clinical expression in future generations. We are developing sensitive methodology for preimplantation diagnosis of the fragile X bearing chromosome, based on detection of polymorphisms in tightly-linked microsatellite sequences. Use of these flanking markers allows tracking of specific X chromosomes in samples consisting of single copies of target DNA, circumvents technical difficulties associated with direct amplification of the CGG repeats, and avoids uncertainties concerning the length of the CGG repetitive tracts or activation status of the fragile X chromosome.

SIZING FRAGILE X PREMUTATIONS AND NORMAL ALLELES WITH NESTED PCR

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SUMMARY

The number of CGG repeats at the fragile X locus is related to the potential for expansion in the female germ line, and may be related to severity of clinical symptoms. Southern blots allow rough sizing of full mutations, premutations and normal alleles, but do not allow precise determination of the CGG repeat number. We have developed a nested PCR-based sizing procedure that routinely provides strong signals for clinical evaluation of CGG repeat number for alleles ranging in size from common normal range of about 29 repeats (or fewer) up to premutations containing about 100 repeats, starting with only 3 nanograms of genomic DNA purified from peripheral blood. PCR was performed by modifications of the method of Fu et al. (1991, *Cell*, 67:1047). Substantial modifications include design of optimized nested primers, initial alkaline denaturation (1992, *Biotechniques*, 12:504), replacement of DMSO by glycerol (1990, *Amplifications*, 5:16), and increased PCR product specific activity. This procedure complements Southern analysis, and should allow routine clinical evaluation of alleles in the normal-to-premutation size range.

IV. Social Concerns

SCREENING FOR THE FRAGILE X SYNDROME

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The fragile X syndrome is a type of X-linked mental retardation which is found to be one of the most common forms of identified mental retardation, second only to Down syndrome. Due to the recent identification of the fragile X mutation, a diagnostic test has been developed which is accurate and inexpensive. Because of the availability of this new diagnostic test in addition to the high frequency and seriousness of this condition, it is timely to consider population screening for the fragile X syndrome.

Screening for the fragile X syndrome at a population level is not to be confused with screening for affected and carrier individuals in a family with a positive family history. Rather, it requires the testing of all individuals in a designated population for the fragile X syndrome. Thus, mass screening is a public health activity and must be evaluated by the community at large, as it impacts the general population.

There are at least five issues that must be considered prior to implementing any genetic screening program. First, it must be decided whether the genetic disorder has a significant impact on individuals and their families. For the fragile X syndrome, seriousness of the condition is related to the mental impairment resulting from the fragile X mutation and to the risk of transmitting the gene to future generations. Furthermore, the frequency of the fragile X mutation must be assessed to determine if the mutation impacts the general population. Although an accurate estimate of the frequency has not been obtained as yet, it is known that the fragile X syndrome is the most common form of inherited mental retardation. Even if a condition is found to significantly impact the general population, screening should be implemented only when the benefits of diagnosis outweigh the drawbacks. Thus, the advantages and disadvantages of an early diagnosis of the fragile X syndrome must be weighed to determine whether obtaining fragile X diagnosis warrants undergoing the discomforts and risks resulting from population screening.

Once it is established that the fragile X syndrome is a significant genetic condition in the population, the choice of a diagnostic test for mass screening purposes must be made. A test used for screening must be accurate and inexpensive. Most importantly, a quality control program must be feasible for the screening test to ensure that all individuals have access to high-quality testing.

Next, the feasibility of the screening program must be assessed. To re-state the point, this assessment must be conducted not only by geneticist, physicians, and economists but, more importantly, by the general community who need to understand the implications of the possible outcomes of testing. Feasibility depends on: 1) the acceptability of the program by the people to be screened, 2) the possible number of cases identified in the targeted population and the ability to follow-up such cases, and 3) the cost-effectiveness of the program. Modell (1991) has emphasized the importance of developing a new approach to judge the cost-effectiveness of a genetic screening program. He states too often the cost of a screening program is described in terms of the dollars saved because the birth of an affected child is prevented. This implies the primary goal of the screening program is to prevent affected births. Instead, the goal of genetic screening is to provide informed choices for couples and individuals at risk and to provide supportive care for affected individuals and their families. As stated by Turner et al. (1992), the ultimate goal of a screening program is to allow individuals who carry the fragile X gene to have the same number of liveborn children they would wish if they were not carrying the gene.

The feasibility of a screening program depends on the population designated. There are advantages and disadvantages which must be weighed for each population. Possibilities include screening 1)pregnancies, 2) newborns, 3) school-aged children or 4) women of reproductive age. If it is determined the frequency of fragile X gene carriers does not warrant screening the general population, it may be possible to screen individuals at high risk. For example, it may be more feasible to screen children in special education classes than to screen all school-age children.

The fourth issue is one that applies to all medical testing, and that is, who has access to results. This issue is important for a genetic condition because a positive result affects all family members, not only the identified gene carrier. For those countries with private health coverage, the adverse effect of being identified as a gene carrier has already been discovered by some; that is, a small number of individuals have lost their health coverage or have not been able to obtain new coverage because of their status as a fragile X gene carrier. This issue will become more significant as additional tests become available to identify genetic disorders. Informed consent for testing and for disseminating results must occur at all levels of the screening process.

Resources must be in place prior to implementation of any screening program. Most importantly, education concerning the fragile X syndrome, as well as genetic conditions in general, must occur at a public level. Furthermore, the screening program should be established so that all people to be screened have access to high-quality testing and follow-up services including medical, educational and family support. Lastly, the funding mechanism for the screening program must be identified. Costs of the program may be incurred by individuals themselves, insurance companies, taxes or through other mechanisms. Again, the advantages and disadvantages of each funding mechanism must be assessed.

In summary, screening for the fragile X syndrome at a population level is an important public health consideration that must be seriously evaluated prior to any program development. To begin this evaluation, a short questionnaire concerning implementing a fragile X screening program was given to attendees at the Third International Fragile X Conference. About 28% (n=88) of the participants of the meeting completed the questionnaire. The majority of respondents were parents of an affected child or medical and science professionals. Seventy percent were female and 75% were between the ages of 31 and 50.

In general, 75% of the respondents thought there is a need for mass screening of the fragile X syndrome. Respondents were asked to rank which target population would be most feasible to screen: 1) women considering pregnancy, 2) pregnancies, 3) newborns, 4) school-age children or 5) school-age children with special needs. Forty-six percent chose women considering pregnancy as their first choice. There was no consensus among respondents regarding their biggest concern about mass screening. Choices included lack of specific treatment for the fragile X syndrome, stigmatization due to carrier status, confidentiality of results, and lack of understanding about genetic disorders by the general public. Lack of understanding about genetics generated more first choice selections; however, many felt that confidentiality and stigmatization may add to the negative outcomes of screening. Ambiguous results may also add to the burden of screening and may be a consideration for fragile X testing at this point in time. Thus, respondents were asked if they would want to be informed given an ambiguous result and there was general agreement to be informed, despite uncertainty of diagnosis. Lastly, the issue of funding was raised and 62% of respondents thought that taxes should be used to fund a screening program. In addition, about 50% thought insurance was an acceptable means for payment.

This type of evaluation is one example of the type of community assessment that needs to be conducted to establish the necessity and feasibility of screening for the fragile X syndrome. In addition, further basic research in the area of fragile X gene expression and the mutational mechanism is needed to provide better prognostic testing and risk assessment. Lastly, pilot screening programs should be established to resolve unanticipated problems prior to screening on a population level.

ACKNOWLEDGEMENTS

This work is supported by grant HD27801 from the National Institutes of Health and by an award from the University Research Committee of Emory University.

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THE ECONOMIC IMPACT OF THE FRAGILE X SYNDROME ON THE STATE OF COLORADO

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INTRODUCTION

Fragile X syndrome (fra X) is the most common inherited form of mental retardation known. Inherited in an X-linked fashion, fra X can cause intellectual deficits ranging from subtle learning disabilities to severe mental retardation and autism. The incidence of this disorder is approximately 1 per 1000. Several epidemiological studies have surveyed retarded populations to obtain these figures. Herbst and Miller (1980) estimated the prevalence of fragile X syndrome in males to be 0.92/1000 when surveying British Columbia. Perhaps the best survey so far was done in Coventry, England, where significantly retarded individuals were cytogenetically screened for fragile X (Webb, et al., 1986). They found that 0.73/1000 males in the general population are retarded because of fragile X syndrome. Females are more commonly less affected. The prevalence of mental retardation in females is approximately 0.6 per 1000 in the general population (Webb, et al., 1986). However, the carrier rate for this abnormal gene, which includes unaffected and learning disabled individuals, is calculated to be as high as 1 in 500 to 1 in 700 individuals in the general population (Opitz 1986). In addition to mental impairment, fragile X syndrome is characterized by a group of symptoms, which include physical and behavioral characteristics and speech and language delay.

CLINICAL CHARACTERISTICS OF FRAGILE X SYNDROME

The clinical characteristics of fra X can be divided into 3 major areas: mental impairment, physical features and behavioral characteristics.

Mental Impairment

The majority of males with fra X have some degree of mental impairment combined with speech and language delays. For reasons not clearly understood, approximately 20% of males, who carry the fragile X gene, are intellectually normal. Approximately one-third of females who carry the fragile X gene have some degree of mental impairment. The remaining two-thirds have normal I.Q.'s but may have subtle learning disabilities (Chronister et al., 1991).

Physical Features

"Individuals with fra X commonly share a number of distinctive physical features. Adult males usually have a long face, large and/or prominent ears, and large testicles. Younger males and females who carry the gene may also demonstrate some of these characteristics. Abnormalities of connective tissue frequently occur including double-jointed fingers, flat feet and a heart murmur due to a floppy valve (mitral valve prolapse). Physical features may be quite subtle and because of this, many children and adults with fra X appear quite normal.

Behavioral Characteristics

Behavior ranges from socially engaging and friendly to autistic-like behavior and/or rare violent outbursts. Fragile X individuals can become easily overwhelmed by excessive sensory stimuli such as crowds or an irritating noise. Hyperactivity is characteristic of the boys with the syndrome. Poor eye contact, hand flapping, hand biting, and fascination with spinning objects are common autistic like behaviors (Hagerman, 1991).

GENETIC CARRIER STATUS, CYTOGENETIC EXPRESSION, AND CLINICAL DISEASE

Most males having the fragile X gene are mentally retarded and express the fragile X cytogenetically. However 20% may be both phenotypically and cytogenetically normal, and are called non-penetrant transmitting males. Approximately two thirds of females carrying the gene are mentally normal, and of these one third are cytogenetically positive. Of the one third of female carriers who are mentally retarded, 90% are cytogenetically positive but 10% are cytogenetically normal. The discovery of the FMR-1 gene has clarified the molecular basis for the spectrum of involvement from unaffected carriers to fully affected, retarded individuals.

While fragile X syndrome is the most common form of *inherited* mental retardation and is second only to Down syndrome as a cause of mental retardation resulting from a genetic defect (Rocky Mountain News, 1991), the extreme diversity of symptomology makes clear diagnosis difficult in some patients. As a result, most data tends to be aggregated into a more general grouping of "developmentally disabled" or another such category. We found very early in our research that throughout the medical community, as well as among the care-providing community, the historical lack of a clearly differentiated diagnosis has led these professionals to aggregate the diagnoses into other more general categories. This is done to expedite reporting to Medicare/Medicaid and state agencies responsible for the care of the retarded community. This has caused extreme difficulty for our research team in the gathering of data.

The methodology for accounting for this tendency will be discussed in the next section. Suffice it to say that every attempt has been made to separate the relevant data into meaningful categories. Frustration with this problem has been expressed by state and federal program administrators, group home care providers, fragile X researchers, educators, insurance providers and hospital administrators alike. As awareness of the pervasiveness of fragile X grows, the need for aggressive diagnostic efforts and more accurate reporting is becoming more and more evident. We are in contact with several research groups throughout the country, and in every case, this lack of disaggregated data has slowed and complicated the research process tremendously. There is a very clear need for a major educational campaign directed toward the general medical community to increase awareness and accurate diagnosis of fragile X related dysfunction. The nature of fragile X and the wide range of symptoms will make this a difficult and on-going process. However, with the newer DNA diagnostic tools (see Diagnosis section of these proceedings) and with an educational process concentrating on clear identification of the symptomology related to fragile X, a clearer body of data should become readily available, making studies of this nature much more streamlined and accurate.

The focus of this study has been narrowly defined to be the economic impact of fragile X on the State of Colorado. Starting with costs to the individual families over and above "normal and ordinary" costs of child rearing and ending with the costs related to providing institutional care and the state and federal dollars expended on educational and medical care for fragile X sufferers, we have attempted to identify the *total cost* of caring for this group of individuals. Once more, due to the problem of aggregated information, the separation of fragile X-specific costs has been a tedious and time-consuming process. Nevertheless, the importance of a true economic analysis based upon a thorough accounting of costs cannot be understated. It is our hope that by providing at least the beginnings of this type of analysis, a picture of the true economic impact of fragile X will begin to emerge.

METHODOLOGY

Information Gathering

The personal family data were gathered primarily through the use of a carefully designed questionnaire intended to identify fragile X specific costs and separate these costs from the normal and ordinary costs of child rearing. The surveys were sent out to a list of 134 families provided by the fragile X clinic of Children's Hospital, and followed up with telephone interviews to expand and clarify the answers we received. Due to the extremely small population of the survey group, no statistical significance could be assigned. However, average values are presented, which we feel should give a fairly concise picture of *average costs* for fragile X families. The surveys were very detailed and therefore some survey error can be expected. Every attempt has been made to eliminate such error in the follow-up telephone interviews. Due to the age of some of the respondents and the time period covered (in some cases, treatment and care began 16 years prior to this survey) and the lack of clear diagnosis at first in the older cases, some costs have had to be estimated. Once more, every attempt has been made to identify these older costs and translate them into 1991 dollars. While these estimates were based upon increased medical care costs throughout the period of treatment, and upon inflation of the dollar, no guarantee can be made as to the absolute accuracy of these estimates.

Findings on state expenditures were primarily gathered from telephone interviews from the various state agencies involved. Once more, our research team ran into the same problem of aggregated data and a general lack of knowledge of the fragile X syndrome specifically. We have worked very closely with the various agencies in making every attempt to identify and isolate fragile X-specific data. In some cases, this data had to be generated by assuming an average percentage of fragile X syndrome as a percentage of the total developmentally disabled population. In attempting to gather information on Medicare/Medicaid payments, once more the data tended to be grouped into more general categories, with very little specific information dedicated to and identified as fragile X related.

Information on group home care and state supported day care programs was gathered through direct telephone interviews with the directors of such homes wherever possible. In some cases, staff personnel supplied the required information. While we have every reason to believe the data given is reliable, we were unable to independently verify the information. In many cases, payments received by the various care providers are mandated and controlled by the legislature. In these cases, very reliable data were obtained. Costs of care have been broken down to cost per individual to maintain accuracy. The total number of fragile X clients was difficult to determine due to the above mentioned tendency to report all cases in a more general category. In many cases, a direct fragile X diagnosis was possible only where the group homes had the benefit of working with a specialist with fragile X experience. There may be many more cases that could be attributed to fragile X that simply have not been identified by genetic screening or other medically reliable diagnoses. At this point, the need for more specific reporting, especially in the case of fragile X, is becoming recognized and more reliable data should be available in the near future.

The Colorado primary education system is mandated to provide special education for those students tested and identified as learning disabled. This identification seldom goes beyond the simple identification of a learning disability, and rarely specifically identifies causality (mental retardation, fragile X, psychological, and/or functional disabilities, etc.). Very little data was available on the true cost of providing special education for developmentally disabled students.

Analysis

Comparative analysis was completed in most cases by averaging the identified costs of all respondents. Any deviation from this methodology will be clearly identified in the appropriate section. While the number of families responding to the survey was relatively small, we are confident that they present a fair representation of the average costs incurred by families affected by fragile X. In each of the surveys, we identified the total number of fragile X sufferers and allocated costs as specifically as possible, depending on the degree of disability suffered by the individuals in question. Specialized needs highly specific to any one individual not representing the sample as a whole have been isolated. In any case where a family has exercised an option for higher cost private care as opposed to publicly available assistance, we have attempted to adjust the figures to be more representative of the norm. However, in any case where the public assistance was deemed to be inferior or insufficient to the individual needs of the fragile X sufferer, the figures were left as reported. All extrapolation of data from federal and state reports was done using the best data available as to incidence of fragile X per unit of the general population.

FINDINGS ON PERSONAL FAMILY DATA

Individual family data were gathered through the use of a focused survey intended to isolate fragile X specific costs. One hundred twenty-three surveys were sent out, with 54 returned, for a response of 44%. Due to the low number of returns, in all cases averaging has been used to present an aggregate picture of the "average" family affected by fragile X. We have taken into account the number of sufferers in each family and have allocated costs proportionally, according to the degree each sufferer was affected by the syndrome.

General Family Information

Family Composition

Each family had an average of 3.32 members with 1.65 members in each family affected by fragile X, for a total of 49.55% of family members affected. There were approximately .81 members of the immediate family no longer living in the house. This included grown children, ex-spouses, and other dependents. Eighty-eight percent of those families responding reported having other relatives, not part of the immediate family, who have been diagnosed with fragile X.

Income Information

Of those families reporting, 19.4% had incomes less than $10,000 annually, 6.1% had incomes between $10-20,000, 6.1% had incomes between $20-30,000, 16.1% had incomes between $30-40,000, 16.1% had incomes between $40-50,000, and 35.5% had incomes greater than $50,000 annually. In aggregate, 31.6% had reported incomes less than $30,000 annually and 67.7% had incomes greater than $30,000.

The weighting of the income ranges toward the upper end of the scale may be attributable to two factors: 1) in those families with greater total incomes, one spouse was able to be at home as the primary care provider and therefore had more time to fill out the survey, 2) in those families in the lower half of the scale, there tended to be a single care provider who may have been affected with the fragile X syndrome, and in many cases had at least a partial learning disability or other overriding circumstances limiting the ability to fill out the survey. In this second case, additional telephone support was required to assist in the completion of the surveys.

Family History

Of those families responding, approximately 17.7% reported a divorce that could be directly attributed to the difficulties of having a fragile X sufferer in the family. Approximately 83.3% reported receiving no financial contribution from the absent spouse. In almost every case where a divorce was reported, the remaining spouse relied on some type of public assistance for both basic survival and fragile X-related health care.

Individual Family Medical Costs

Diagnostic Costs

Of those families responding, 66.7% received an incorrect diagnosis and incurred medical expenses for problems later determined to be fragile X related. On average, there were 4.9 incorrect diagnoses per responding family. The average cost for incorrect diagnoses in these families was $2,095.83.

Prenatal Screening

Of those families responding, only 16.1% had prenatal screening (many of the families reported that prenatal screening was not available at the time of the pregnancy). Average costs for prenatal screening was $1,733.33 per family. In 50% of the families screened, fragile X was detected. Only one family reported fetal blood tests. Cost for that testing was approximately $1,600.00 and had a positive result (fetus is fragile X-affected; the degree to which the fetus is affected cannot be determined at this point).

Medical Costs Related to fragile X

Of those families responding, average costs for hospital fees, including diagnosis, treatments, check-ups, etc., averaged $487.75 annually per family. Doctors' fees, including home, office, or hospital visits, averaged $482.50 per family. Costs for prescription medicines averaged $191.50 per family annually. Therapy costs were broken down into three major areas: physical therapy averaged $1,583.33 per family; psychological therapy and counseling averaged $678.57 per family; and occupational therapy averaged $1,485.71 per family annually.

Dental costs were included in the survey, but every attempt was made to limit such costs to fragile X-specific care (tooth grinding and some displacement are common symptoms of fragile X, particularly in males). Average costs per family for dental care is $377.50 annually.

Specialist care, including dieticians and other required trained specialists, averaged

$3,000.00 annually per family. Costs for specialized medical equipment, including orthopedic braces, crutches, wheelchairs, or other such devices, averaged $198.00 per family. Many families reported a special need for speech therapy. On average, each family spent $1,860.00 annually on these costs.

Other miscellaneous medical costs averaged $981.50 per family.

In total, the average family in our survey could be expected to spend approximately $11,326.36 annually for fragile X-specific care. This expense is over and above the "normal and ordinary" costs of child rearing. Given the normal pattern of the fragile X sufferer remaining with the family through the 18th year, this means that a fragile X family will spend on average $203,874.48 on fragile X specific care, per person affected during this time.

Only 6.5% of the families responding reported a relocation of their family in order to obtain specialized fragile X care. However, many of the families reported that they were unable to relocate for job advancement or other family advantages. We were unable to determine possible income loss due to the inability to relocate for medical reasons.

Legal Costs Relating to fragile X

The most common legal cost reported was related to the parents retaining legal guardianship when fragile X children reached majority age (18 years in Colorado). In most cases, this was done so that the parent could have a continuing input in the care and treatment of severely disabled children. Average cost per family for legal fees was $5,367.55. These costs tended to be one-time rather than on-going and therefore have not been annualized.

Insurance Costs Relating to fragile X

Of those families responding, 84.4% were covered by some type of insured health care plan. In most cases, this plan was at least partially paid for by the employer of the primary care provider. Of those responding, the reported average annual premiums for health care coverage was $1,246.34. It must be noted that only 57.1% reported that fragile X costs were covered. One family reported being denied coverage due to a "pre-existing condition." In a report of the Colorado Department of Regulatory Agencies, Division of Insurance, dated September 1990, nervous/mental disorders were listed as the fourth leading reason for rejecting a medically underwritten *group* and the third leading reason for declining to insure an *individual* (Dept. of Regulatory Agencies, 1990).

Modifications to the Physical Environment

In only one case were any costs incurred for modifications to the home for fragile X-specific care. The primary reason for this modification was to allow for the installation of a hospital bed, and the approximate cost for this modification was $5,000.00. No modifications to the family car were reported.

In the one case where specialized medical furniture was required, the aggregate costs of this furniture and other specialized equipment amounted to $20,000.00. This was a highly specific case where the fragile X sufferer was severely disturbed and the choice was made to care for the individual in the home rather than opting for institutional care.

Special Education Costs

Of the families responding, 86.2% reported the need for special education programs for their fragile X sufferers. In 96% of those families responding, care was provided through the public school system or other government agencies. Twenty-eight percent reported costs averaging $1,724.14 per family (total includes transportation to and from these programs, any specialized supplies required, and in some cases minor registration fees). These fees were not annualized, but represent an aggregate total.

Of those families reporting the use of public school programs, 21% had to make special arrangements for that care (these programs were not freely offered, but the burden was on the parents of the sufferer to seek the special program).

Childcare and In-home Care Costs

Only 15% of those families responding reported the need for specialized care over and above normal childcare. The cost of this care averaged $4.67 per hour. In one case, specialized nursing care was required at $45.00 per hour.

Eighteen percent of the families reported the need for special respite care for family members affected by fragile X. There are many creative ways of providing this respite care, including the exchange of time among various family members. Only one family reported using a specialized program at a cost of $6.00 per hour.

Of those families responding, 18% reported that one of the adult members of the family is prevented from working in order to provide care to fragile X family members. An additional 17% reported that some of the wage earners in the family regularly lose work time to provide fragile X related care. The variability of the number of hours lost prevented any accurate determination of total lost hours, therefore no average cost could be assigned.

In many cases, families made special arrangements in order to make ends meet, i.e., husband and wife worked different shifts to accommodate care, one or both parents worked part-time, etc.

Miscellaneous Costs Relating to fragile X

Nine percent of the families reported that their fragile X sufferers required a special diet. Average costs for dietician services were $40.00 per hour. Data on the costs of special foods over and above normal food costs were incomplete and are not reported in this study.

Twenty-three percent of the families reported the need for special emotional support for family members not affected with fragile X. Here again, the wide variability of the costs for counseling was such that it prevented any accurate accounting for average costs. However, some costs reported were from a high of $600.00 per month to a low of $25.00 per month, and/or $80.00 per hour for counseling.

Summary of Findings on Personal Family Data

In summarizing the financial burden on the average fragile X affected family, we are making the assumption the normal pattern for these families is to care for the fragile X sufferer from birth to 18 years, and then to transfer responsibility for care to a state sponsored program for the developmentally disabled appropriate to the degree of disability of the fragile X sufferer. These totals are based strictly on an average profile garnered from the surveys returned. We feel that they represent a fairly accurate average cost of care for each family affected by fragile X.

Total fragile X Specific Cost of Care (Birth to 18 Years)

Medical Costs & Therapy	$203,874.48
Diagnosis	2,095.83
Pre-Natal Screening	1,733.33
Legal Costs	5,367.55
Insurance	22,434.12
Special Education	1,724.14
Total	**$237,229.45**

FINDINGS ON PUBLIC EXPENDITURES

The findings on public expenditures are strongly divided according to the degree of disability of the fragile X client. Here again, care is normally provided from 18 until death. Medical literature suggests that fragile X sufferers have no significant loss of longevity. To that end, we will base our total cost estimates on an average life span of 72 years minus the 18 years that care is normally provided by the family. Please note that during that 18 years, a wide range of public assistance is provided depending on the individual family situation. This public assistance will take the form of welfare, Aid to Families with Dependent Children (AFDC), Medicare/Medicaid, special education programs, and publicly sponsored support groups.

In the case of severely retarded and/or violent sufferers, Medicaid pays a daily rate of $232.92 per patient. According to information provided by Ridge Home, a.k.a. Wheatridge Regional Center, their average length of stay for fragile X sufferers to date is 22 years. This would indicate that on average, Medicaid has paid $1,870,347.60 per patient. Ridge Home currently has six remaining patients clearly identified as fragile X although other patients may be fragile X affected, but not specifically diagnosed as such. Therefore, this average can be expected to increase, depending on the life span of the six remaining patients. Based on our average life span (72 years, minus the 18 year normal family care, or 54 years total) we can expect a total cost of $4,590,853.20 per fragile X patient who is severely retarded and who requires institutional care.

For patients who require full supervision but who can function in a sheltered workshop environment, Johnstone Developmental Center reports the following amounts paid to benefit their fragile X patients:

Social Services	
For psychological counseling, room and board, and dental work.	$ 85.00 per day
For personal use	1.30 per day
Department of Developmental Disabilities	
Sheltered workshop	45.00 per day
Medicaid	
Prescriptions	.33 per day
Any other medical care as needed	Variable
Daily Total	$ 131.63 per day
Lifetime Total Per Patient	**$ 2,594,427.30 (54 years)**

For the less severely affected fragile X sufferers, state run residential care programs report the following costs:

RESIDENTIAL CARE

Degree Affected	Daily	Life
Full supervision (severely handicapped)	$ 87.00	$ 1,714,770.00
Moderate supervision	58.00	1,143,180.00
Intensive adult resident (40-50 hours per week supervision)	34.00	670,140.00

DAY PROGRAMS (State mandated maximum of 1,439 hours per year; approximately 27.67 hours per week)

Degree Affected		Hourly	Yearly	Life-Time
Severely handicapped (with "basic skills")	$	4.15	5,971.85	322,479.90
Capable of working		3.00	4,317.00	233,118.00

The approximate break-down of funding sources for the above programs is as follows:

Medicare/Medicaid	70-80%
Colorado State General Fund	20-25%, depending on program
City & County	0-5%, depending on program

Across the board averages for all programs combined within the State of Colorado suggest a $1,609,852.63 average cost of care per fragile X patient for ages 18-72.

COMBINED TOTAL AVERAGE COST OF CARE FOR THE FRAGILE X SUFFERER

Based on our findings of an average normal life of 72 years, wherein the first 18 years of care is covered by the family, and the remaining 54 years is covered by some form of state program, the average combined cost of care for each fragile X sufferer within the state is as follows:

Family contribution through age 18	$ 237,229.45
State contribution through age 72	1,609,852.63
Total combined cost of care	**$1,847,082.08**

These figures represent a complete *averaging* of all fragile X related costs of care based on our surveys. While there will be wide variation in *individual* costs, we feel this figure will be very close to the true average cost of care, based on across-the-board averages for all fragile X sufferers. It should be noted these figures correlate very closely to the totals derived by the

New York State Office of Mental Retardation and Developmental Disabilities. According to that office, the average lifetime cost of caring for a mentally retarded individual is $2 million ($1 million - $4 million depending on the level of care required) (Nalin et al., 1991). This) (Nalin et al., supports the findings of Turner, et al. (1986) who reported a "conservative estimate" of over $1 million Australian over a lifetime of care.

The overall financial implications of these findings for the state of Colorado are enormous. Given a 1990 population of 3,294,394 and an average male penetrance of 1:1000 and a female penetrance of 1:2000 for retarded individuals (these figures are the most generally accepted penetrance rates for fragile X-related retardation [Webb et al., 1986]), this implies a total fragile X-related retarded population of 2,471 individuals. **The average lifetime cost of state provided care (ages 18 - 72) for these individuals alone can be expected to be: $3,977,945,848.73, or about $73,665,663.87 annually.** Obviously this amount will increase as the population base of Colorado expands.

Nationally, given a population of 248,709,873 (1990 census data) and the same penetrance rates, the fragile X population would be approximately 186,592 fragile X-retarded individuals increasing by 3,150 individuals annually. If we use a conservative estimate of $1.5 million lifetime cost of care for these individuals, we come up with a current burden of approximately $280 billion 1990 dollars. This expenditure will follow national population trends and will be proportional to changes in the overall population.

To get a completely accurate picture of the true cost, reporting procedures and identification of fragile X syndrome sufferers will need to improve. This will require a massive educational program to teach health care professionals about fragile X and how to diagnose it. This process is underway now and we feel that with continued diagnostic effort, more accurate data should become available within approximately five years.

CONCLUSIONS

As we have repeated many times throughout this report, the difficulty in gathering accurate data for fragile X syndrome cost analysis has been complicated tremendously by the lack of accurate identification of fragile X patients. Although the classic patient is retarded, this gene causes significant emotional problems and learning disabilities in mildly affected carriers of normal I.Q. At this time there is no way to estimate the costs of milder degrees of dysfunction. This report is based on the costs of retarded individuals only. In addition, most groupings of mental retardation have not yet designated a classification for fragile X persons. As a result, the fragile X patient tends to be categorized into other better known groups (i.e., developmentally disabled, autistic, etc.). The cytogenetic tests for a clear identification of affected fragile X persons cost approximately $450.00 per patient. This represents only 0.00022% of the life-time costs of caring for a fragile X individual. Newer DNA diagnostic testing is approximately half of the cytogenetic costs and will probably become less expensive utilizing PCR technology in the future (see Brown et al., these proceedings). Although treatment exists, including medication and therapy, there is no cure as yet. However, since the recurrence risk is so high, genetic counseling can affect reproductive decisions and potentially decrease the incidence of this disorder. Individuals must be identified before counseling can be given to the multiple carriers in a family who are at high risk of having fragile X children. The cost of this diagnostic testing will be orders of magnitude below the cost of caring for each individual fragile X person. If a counseling and awareness program resulted in a 50% reduction of the number of fragile X-affected births, the savings to the state would be tremendous.

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ETHICAL ISSUES IN THE PROVISION OF GENETIC SERVICES: THE MULTIDIMENSIONAL IMPACT OF THE HUMAN GENOME INITIATIVE

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The re-examination of Darwin's "survival of the fittest" and Mendel's description of the inheritance of traits to "factors" (genes) has burgeoned in the twentieth century with the effort of the human genome initiative. Estimates of the genetic impact on health care delivery show that the genetic basis for hospital admissions range from 25%-54% (Kelly, 1986). Furthermore, birth defects are ascribed as an underlying or contributing cause of death for approximately 25% of reported infant deaths (MMWR, 1989). Explanations for the increasing visibility of genetic conditions within the field of health care have been attributed to the advent of expanding genetic knowledge and technical expertise, the increasing awareness of the diagnoses of genetic diseases and by the disproportionate allocation of resources genetic diseases consume due to their chronic nature (Kelly, 1986) and the expense of the technology.

So how has the present technology reshaped the questions Darwin and Mendel posed? Has the understanding of what is "fit" and "normal" changed? Under what circumstances and from whose perspectives are decisions valued as acceptable or inappropriate? These questions carry an assumption of explicitness which the field of genetics finds uncertain. The field of genetics is not based on absolute certainty but rather on probabilities. Thus, the issue does not necessarily lie in what can and cannot be done, but rather what the boundaries of tolerance are for the outcomes of these probabilities. Therefore, a more apropos query should focus on how these probabilities are disseminated and under what circumstances.

To gain a clearer understanding of the genetic decision-making process, a synopsis of three distinct but related areas must be examined. First, the underlying concepts of autonomy, informed consent, confidentiality and privacy will be elucidated. The application of these concepts by individuals, families and professionals who seek and provide genetic services will demonstrate that the decision-making process for each of the groups are guided by their own personal histories and professional proclivities. Second, the social implications and consequences of genetic decision-making will be examined through the application of genetic testing and the utilization of genetic information. And third, genetic research and clinical applications will be scrutinized within the realm of an international perspective acknowledging scientific, social, political and significant cultural influences.

GENETIC DECISION-MAKING

Several ethical principles are relied upon in the genetic decision-making process:

autonomy with informed consent and confidentiality and beneficence and nonmaleficence under the auspices of justice.

From the Greek autos (self) and nomos (governance), autonomy accords self-determination without physical or psychological coercion or constraints by others. The understanding of self-governance, of being one's own person, concedes an individual's ability in considering choices given as well as the capability of operating on the basis of such intentions (Beauchamp & Childress, 1983).

Informed consent serves to promote individual autonomy (Beauchamp & Childress, 1983). Informed consent fundamentally values self-determination and dictates that individuals must be given information concerning risks and benefits as well as be allowed to make a choice of acceptance or denial. Informed consent implies a ***voluntary*** agreement resulting from a person's ability to exercise free will and power of choice that is unfettered of ulterior forms of constraint. Thus, informed consent requires sufficient knowledge and comprehension of the subject matter involved to enable a person to make an understood, informed decision (Beauchamp & Childress, 1983).

Confidentiality expresses the right to self-determination in contexts involving personal information. Here, maintaining confidence of information about an individual is justified by the right of privacy (Jonsen et al., 1986). Derived from self-determination, confidentiality and privacy are based on autonomy. Here, confidentiality and the right to privacy declare the prerogative to self-determination in situations involving personal information. Authority is given to the individual's interest in determining what will be known about him or her (Beauchamp & Childress, 1983).

Justice is the action towards a person that is due and speaks in the voice of fairness. Furthermore, in distributive justice, fairness addresses the rationalized distribution of benefits and burdens in society that are dictated within the framework of moral, legal and cultural rules that are implicitly and/or explicitly implied (Beauchamp & Childress, 1983).

But how are justice and fairness applied? Through the auspices of beneficence, justice is found in conduct that requires treating persons autonomously through positive acts and assisting in the prevention and removal of harm and harmful conditions (Beauchamp & Childress, 1983). Conversely, nonmaleficence addresses the issues of not inflicting harm onto others and encompasses not only actual harm but also risks of harm (Beauchamp & Childress, 1983).

The application of these ethical principles under the auspices of beneficence and/or nonmaleficence are used by individuals, families, professionals and society at large in a competing fashion, where one principle may be more salient and valued by one participant compared to another participant's perspective. Where difficulties in decision-making occur are when the ethical principles (reflecting values and agendas) employed by one participant are not deemed as valuable or salient by the other participants in the process of ethical decision-making.

The complexity of ethical decision-making and the distinct saliencies of particular principles for each participant can be more clearly illustrated by examining the ethical principles valued by a single participant or group. Although each participant (individuals, families, professionals, society) may value and hold a particular ethical principle as more salient, the following example also demonstrates the variability of these saliencies espoused in defending a decision. In a study by Wertz and Fletcher (1990), an attempt was made to analyze the approaches to ethical problems in the clinical practice of medical genetics. The working assumptions for the study included respecting parental or patient autonomy, an ethical obligation to prevent suffering caused by genetic disease, and the obligation to disclose full test results. Case vignettes with competing ethical principles were presented to medical geneticist in 18 countries. In particular, a vignette involving conflict between the ethical principles of the professionals duty to preserve patient confidentiality and justice *and* the duty to warn third parties (relatives at risk for genetic disorders) about potential harm had no consensus. Approximately 60% of medical geneticists believed that duties to relatives at risk should override the patient's desire for confidentiality, 29% would seek out and tell the relatives even if the relatives did not ask for information, 31% would tell the relatives only if the later asked, 27% would preserve confidentiality of the patient, while 12% would refer the matter to the patient's family physician for decision. Not surprisingly, the results reflect a heterogeneity of opinions of what constitutes an ethical decision and why for medical geneticists.

Even so, decisions must also be accounted for from the positions of the individual and the society at large. From the perspective of the client, privacy and autonomy may be the overriding issue for nondisclosure. Sparing other family members to the perceived socially stigmatizing, guilt-inflicted and often blaming nature of genetic information may be the more salient concern and justify withholding genetic information and potential risks. For family members, the more salient issue may involve potential harm to at risk individuals and raises many questions. Do family members have an inherent right to this information? What if family members do not wish to know this genetic information? Should they be informed against their wishes? Conversely, what if a family member is seeking this information for family planning purposes? Who has the authority to give this information?

Additionally, some aspects of the ethical principles that guide our genetic decision-making are seemingly invisible. For instance, the principle of autonomy prescribes the individual having the right to information which may be highly relevant to his/her own health or that of his/her offspring. Conversely, autonomy dictates the right not to know (and is ideally exercised to the full awareness of the risks attached to not knowing). Implicit within the freedom to make an autonomous choice is the option that unwanted information about one's genetic make-up is not thrust onto an individual. Therefore, to pressure an individual or family to utilize all available technology (in the name of a "technological imperative") for risk assessment and prevention, should be avoided and may be considered unethical and contrary to the principle of autonomy.

SOCIAL IMPLICATIONS OF GENETIC DECISION-MAKING

The principles of justice and distributive justice have been at the forefront of social concerns for many within and outside the field of genetics. For example, under the ethical auspices of justice, individuals fear that classes of persons will be separated based on different genetic characteristics possessed by those persons. But this fear is based on previous experiences of the uses and misuses of genetics in the past such as the eugenics movement in the United States and abroad and the previous problems arising from screening programs of sickle-cell trait (Office of Technology Assessment, 1983).

From the perspective of society as a whole, the issue of distributive justice and shared risks may hold more saliency. For example, the insurance industry may argue that due to the limitations imposed in the allocation of resources, reimbursement of services must be selective (Capron, 1990; Gostin, 1991; Murray, 1991; Wilfond & Fost, 1990). Thus, the insurance industry determines who will be insured and for what premium depending on the assessed "risk." But dilemmas exist in the rights to the information. If a company is fiscally responsible for the genetic testing, do they have a right to the results? Furthermore, since this genetic information may be available to a select number of family members, if an uninformed family member has an affected child, where will the responsibility lie for the child's health and health care costs? What price does society pay for not disclosing the results to all family members at risk? Thus, the insurance industry may desire this information to be disclosed to all of the family members at risk regardless of the affected individual's wishes of nondisclosure "for the good of society."

There has also been growing concern about the type of information which has a predictive value (Brandt et al., 1989; Quaid et al., 1989). Although a prima facia case exists for the absolute confidentiality of genetic information, conflicts of interest may arise that need to be considered. For example, an individual whose DNA sample is necessary for DNA linkage studies (Brandt et al., 1989) may choose not to participate in the family study. Here, the issues of justice and of non-disclosure of genetic information become more salient. Should the non-disclosure of genetic information prevail but only up to the point where it would threaten the interests of the individual, or would impinge adversely on the interests of others - family members, fellow employees, the public at large? What is our limit of tolerance?

Thus, one of the most contentious sets of difficulties resulting from a human genome initiative lies in the use of its results. For example, as a by-product of the project, a greater number of diagnostic tests for specific traits and conditions will become available. The scientific and medical communities will receive an increasing variety of screening requests, ranging between those from couples making reproductive decisions (Lippman, 1991) to those from employers planning personnel policies (Murray, 1991). The resurgence of the very personal nature of the information the tests yield will be faced in considering those requests.

One of the more controversial applications of the new genetic technology would be their use in industry and/or organizations to identify individuals who might be occupational (Andrews & Jaeger, 1991; Gostin, 1991) or insurance risks (Murray, 1991; Orentlicher, 1990; Rothstein, 1989). The appropriate use of genetic information will also be raised within the more intimate circle of the nuclear family. For example, are there limits on the traits that parents may decide their children must have? What are the indicators of need for prenatal diagnosis (Lippman, 1991)? Traditionally, these limits have been set at the boundaries of the pathological spectrum yet the boundaries of conditions that might be regarded as pathological are becoming increasingly vague. As genetic markers become available for an increasing range of traits, the ability to identify those markers prenatally will present difficult decisions for clinicians. What levels of disease susceptibility or risk warrant prenatal diagnosis? Furthermore, are prenatal tests for correctable genetic defects or diseases with late onset appropriate?

THE INTERNATIONAL GENETICS EFFORT

The human genome initiative encompasses research efforts, clinical applications, and the utilization of information within an international reality. Ideally, the human genome effort must include all countries even though not all countries have the resources to conduct human genome research and develop new technology (McKenzie, 1987). This will require a concerted effort to transfer technology and knowledge from those countries having this expertise to those who are less well equipped in this area. But many questions continue to exist and have been addressed on a more theoretical level. Should human genome research be conceived as an international effort and to what extent should each nation or research group develop its own independent research agenda? Human genome research stands to benefit the wealthier, more developed countries. Since the more prosperous countries conduct the majority of the research, research priorities may reflect their own national interests and markets. Thus, genetic knowledge may be utilized in those countries that have sponsored the research or are sufficiently wealthy to obtain a share. Are alliances between scientists, corporations and the state (including the military) compromising scientific integrity of human genome research? What impact does existing laws have on the issue of intellectual property in acquiring the proper balance for insuring incentives for research and fostering productive scientific cooperation, mutual progress and humanitarian assistance?

THE PERSONAL IMPACT OF GENETIC KNOWLEDGE

There are individual and personal repercussions of genetic knowledge that must be acknowledged. In many instances, recipients of genetic information feel guilt about carrying a gene which could lead toward disease and/or passing on a disease gene to an offspring. Individual psychological responses including the impact on self-image (Campbell, 1988, Headings, 1979; Marks et al. 1989; Meissen et al., 1991) must not be lost within a "technological imperative." Genetic information can be highly "charged" when given to individuals and couples who have their own unique social contexts, histories and highly personalized interpretations of the data. Thus, individuals may interpret, or misinterpret, genetic information in idiosyncratic ways. Educational and supportive services are therefore a must.

CULTURAL INTEGRITY AND GENETIC SERVICES

The field of genetics must also recognize the impact of cultural and ethnic differences in the provision of genetic services. Within the next century more than half of the total U.S.

population will be racially and/or ethnically visible. Projections by the year 2000 postulate that 1 out of 4 "Americans" will be a member of a racially/ethnically visible group. By the year 2000, the African-American population is expected to grow by 12%, Asian/Pacific Islanders by 22%, Latinos by 21% and Caucasians by 2%. This trend of diversity necessitates training with more sensitivity, greater knowledge and relevant counseling strategies for culturally and ethnically diverse groups. Unfortunately racially and ethnically different groups generally underutilize medical/mental health care facilities compared to their Caucasian counterparts (Sue & Sue, 1990). Some reasons for this behavior can be attributed to cultural values that stigmatize help-seeking behavior. But other reasons for under-utilization may lie in the theoretical underpinnings of genetic services themselves; the services offered may be frequently antagonistic, inappropriate and/or lack the sensitivity to the understanding of the life experiences of the culturally different client.

In many cultures, the identification of disease genes is accompanied by social stigma, even though an individual may be a carrier of a recessive gene or a balanced translocation carrier and be phenotypically normal. Thus, extreme care must be taken in developing genetic services to not create a genetically stigmatized "underclass" who are ashamed by the information and ostracized at home and in the community. For example, in some families, genetic information is not communicated to relatives out of fear that young adults will become unmarriageable (Wang & Marsh, 1992). Thus, the more severe the stigma, the more reluctant health care providers may be in conveying genetic information. Thus, the diagnosis of a high burden disease in a culture which stigmatizes genetic conditions sets the stage for compromising treatment and management, dramatic psychological reactions and disintegration of the family unit. Therefore, attention must focus on the interpersonal and intrapsychic context in which individuals and families are receiving genetic information. Furthermore, concentrated public and psychological education is necessary to combat such attitudes. By acknowledging and understanding (1) cultural conceptual differences of health and illness, (2) treatment expectations, (3) therapeutic alliances, and (4) modes of communication which maybe incongruent to the western perspective of what is appropriate exist, genetic services will be able to more effectively reach the population at large.

CONCLUSION

When examining the ethical issues in decision-making, competing principles must compel the decision-maker to examine the various agendas each participant maintains. What must be examined is to understand that the decision-making process is an arduous one with each participant possessing a different inclination. To ensure that ethical decision-making is realized, is to ensure that the competing perspectives of the individual, family, professional, and society are seen realistically within a more holistic arena respecting political, economic, and cultural differences.

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WRAPUP

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I want to thank all of you for coming to this conference. Thank you for your praise and criticism. We'll use both. It is an impossible task to summarize this conference, but I'd like to leave you with an impression.

I was fortunate to be able to attend the recent genetics and ethics conference where theologians and geneticists shared ideas. The technical and theoretical views were presented, but few people whose lives would be directly affected were present. What is exciting about this conference is that we have the theoretical, the methodology, the practical and all around us families with fragile X urging us for more answers. Sometimes we are pulled in different directions, but all together we move forward. We need each other and together we can find a cure and value each life.

Fmr - 1
neuRon
dnA
heterozyGote
Inactivation
methyLation
nEurotransmitter

Xq 27.3

rebuS
sensorY integration
Network
Diagnosis
paRents
Occupational therapy
faMily
speech-languagE therapy

cyclic amp Cascade
southern blOt
Neuropsychology
Folic acid
allelEs
Research
prEmutations
dephosphorylatioN
CGG
Education

DONATION

The National Fragile X Foundation, as a not-for-profit organization, is dependent upon grants and donations to continue its existence. We feel that our mission; to promote education, support for families and research, is very important and provides a much needed service.

Our long-term goals are: to develop and implement a National/International Fragile X Awareness Campaign, to continue providing the most current, accurate information to families and professionals, to provide support to families with fragile X and to encourage research.

We need your ongoing support to make these goals a reality. Please give generously. All contributions to the National Fragile X Foundation are tax-deductible as allowed by law. Please call (303) 333-6155 or 1-800-688-8765 for more information.

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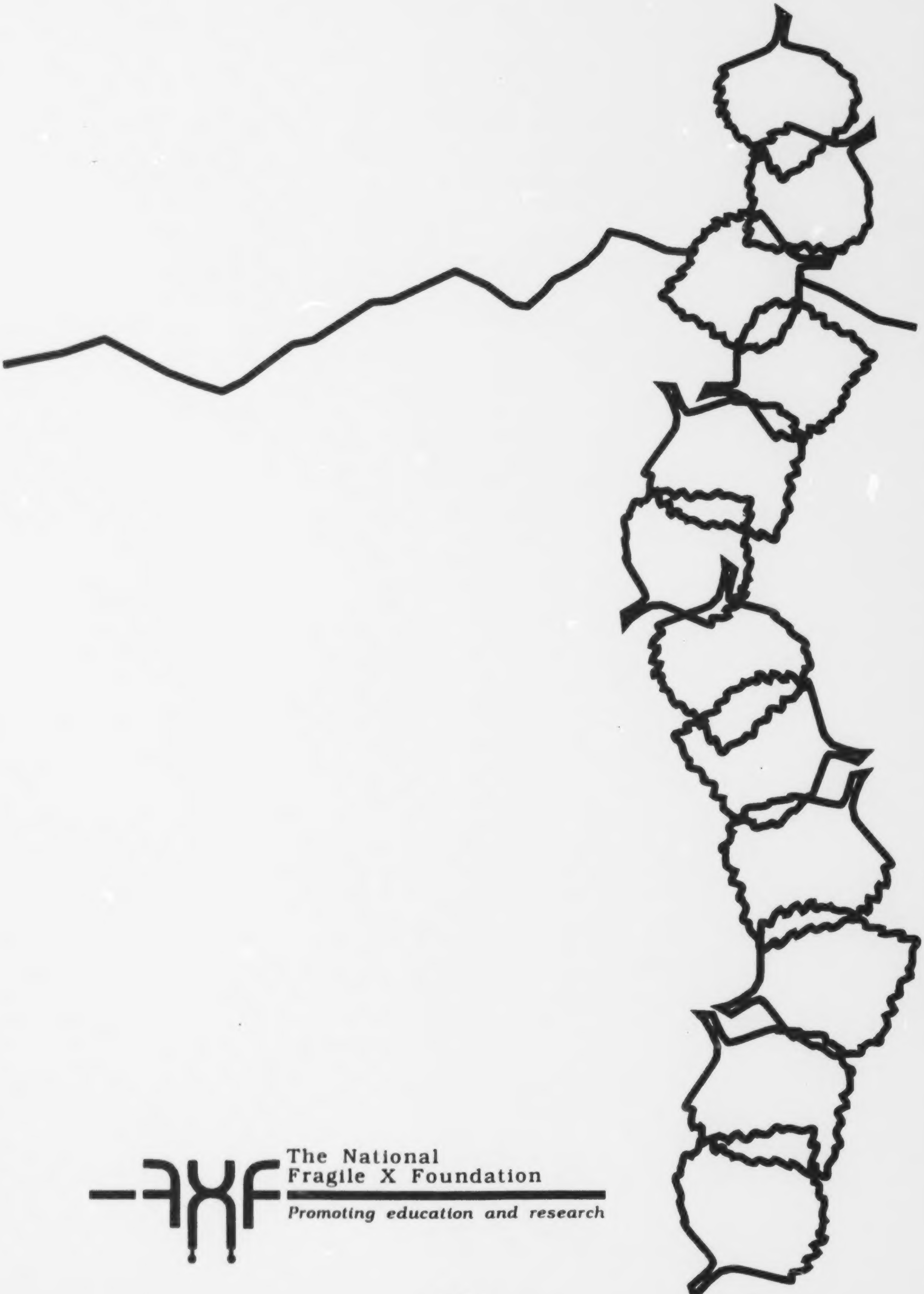

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